An Adeno-Associated Viral Vector for Gene Therapy Delivery to the Peripheral Nervous System

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Background

- Peripheral nerve injuries (PNI) affect many people and are associated with long term debilitating issues for affected patients. Unfortunately, there are no therapies to date that have been shown to reliably restore full clinical function to patients affected by these types of injuries.
- Gene therapy has grown in popularity as a potential treatment strategy for a variety of human diseases, in particular those without known treatments. Gene therapy works by delivering a gene to a cell, which is then turned into gene products by the cell's own machinery (Figure 1). The goal of using gene therapy for PNI would be to increase axon regeneration. This may be done by adding a gene coding for a neurotrophic protein.
- Adeno associated virus (AAV) vectors have been shown to be effective gene therapy delivery candidates based on their safety profile and ability to sustain expression of a transfected gene over extended periods of time. These AAVs come in various serotypes which each have preference for targeting different tissues.
- In particular, the AAV-PhP.S serotype has been shown to have preference for the peripheral nervous system (PNS) making it a promising vector for targeting gene therapy delivery to this region.
- Additionally, the human synapsin 1 (hSyn) promoter has been shown to be an effective promoter for inducing gene expression in neurons.
- However, the extent to which this AAV serotype and promoter combination results in targeted gene expression to neurons in the PNS remains to be evaluated.

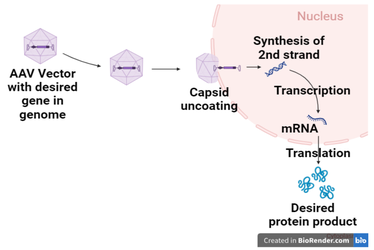


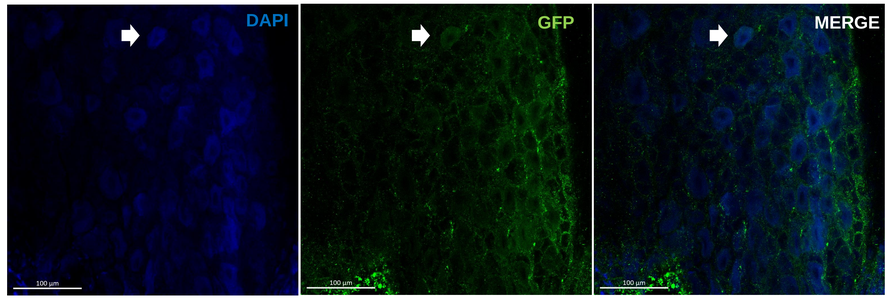
Figure 1: The central dogma of molecular biology is the basis for the gene therapy model. Delivery of a gene with a viral vector will lead to downstream production of a desired gene product using the cell's own machinery.

Aim

- Characterize and quantify the level of gene expression caused by the AAV-PhP.S-hSyn vector construct on DRG tissue after sciatic nerve injection in a rodent model by using an eGFP reporter gene

The AAV-PhP.S-hSyn-eGFP vector effectively transduces the dorsal root ganglion after injection into the sciatic nerve.

LEFT L2



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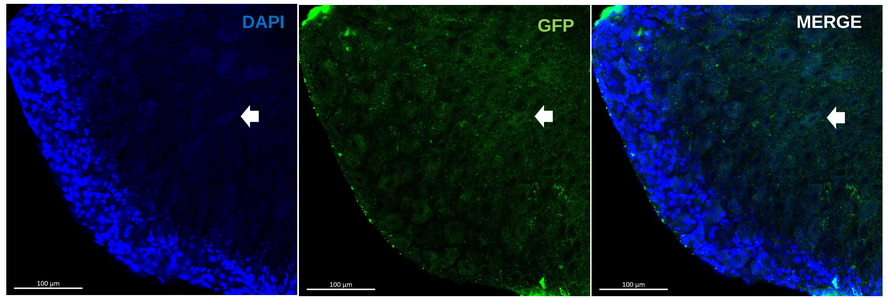
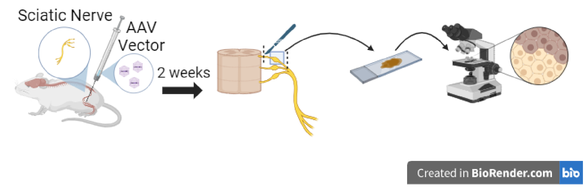


Fluorescent immunostaining against eGFP (green) and DNA marker, DAPI (blue), was done on whole dorsal root ganglions (DRG) from mice that received injection of AAV-PhP.S-hSyn-eGFP. Two representative DRGs from one mouse at different spinal levels are shown in the images. Transduced neurons labeled by eGFP present as circular cell bodies. White arrow: eGFP-stained neuron.

Methods



- AAV-PhP.S-hSyn-eGFP vector was unilaterally injected into the right sciatic nerve of mice
- The vector had a titer of $1.9 \; x \; 10^{13}$ GC/ml and was delivered in 1 ul injections
- Mice were sacrificed, perfused, and dissected after a waiting period of two weeks
- After fixation of the spinal column and cord with paraformaldehyde, dorsal root ganglion (DRG) were dissected
- Fluorescent immunohistochemistry was performed on free floating whole DRG against the eGFP protein
- Microscopy was performed on mounted DRG tissues

Conclusions & future work

- Current treatments for peripheral nerve injury fail to fully restore function. Thus, there is a critical need for regenerative therapies that can restore pre-injury function.
- Here, we characterize the gene expression pattern caused by the AAV-PhP.S-hSyn vector on DRG tissue. The vector construct resulted in gene expression in the DRG at various spinal levels after unilateral injection of vector into the sciatic nerve. The production of a gene therapy vector capable of inducing strong and selective gene expression to the peripheral nervous system is a critical step in making a treatment for peripheral nerve injury.
- Next steps include quantification of the gene expression and determination of its selectivity for neurons to make definitive conclusions about the selectiveness and strength of the gene expression produced by this vector construct. An ideal vector is one that can maintain strong expression in its target area while reducing expression in non-target areas.

Acknowledgements

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- Kügler, S., Kilic, E., & Bähr, M. (2003). Human synapsin 1 gene promoter confers highly neuron-specific long-term transgene expression from an adenoviral vector in the adult rat brain depending on the transduced area. Gene Therapy, 10(4), 337–347. https://doi.org/10.1038/sj.gt.3301905
- Diagrams created with BioRender.com

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