Defining the Tropism of various rAAV serotypes within the Brain Ventricular System

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Introduction

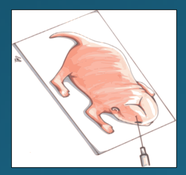
The brain ventricular system consists of four interconnected cavities (ventricles) in the brain. Ventricles are the site cerebrospinal fluid (CSF) production. They contain specialized ependymal cells involved in the production of CSF. Targeting the ependymal cells lining the ventricles is an attractive target for gene therapies producing a secreted protein. We have utilized four different capsid serotypes of rAAV (AAV1, AAV5, AAV6, AAV8) along with four different promoters: strong hybrid promoter (CBA), glial fibrillary acidic protein (GFAP), brain lipid-binding protein (BLBP), and neuroectodermal stem cell maker (Nestin) to generate 16 rAAV vectors that all express EGFP. We observed rAAV tropism within the lateral ventricles by detecting EGFP expression using immunohistochemistry after 15 days of rAAV intraventricular injection into a neonatal mouse (p0).

Hypothesis

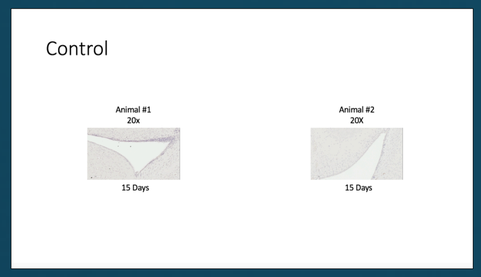
We predicted the rAAV8 with the CBA promoter would result in the most apparent tropism because of its success in transduction of the mouse neonatal brain after the p0 injection.

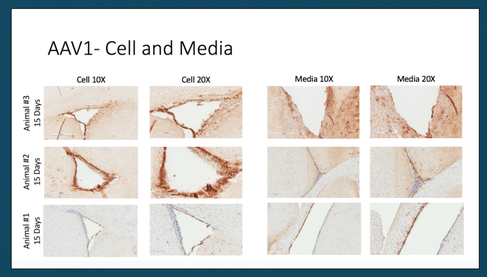
Methodology

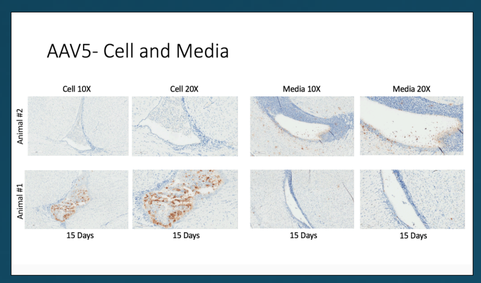
Neonatal mice received a rAAV intraventricular injection. After 15 days, their brains were harvested, sliced, and then mounted on glass microscope slides. After assuring that the ventricles were visible and intact, we observed rAAV tropism within the lateral ventricles by detecting EGFP expression using immunohistochemistry. Four different capsid serotypes of rAAV (AAV1, AAV5, AAV6, AAV8) were utilized along with four different promoters: CBA, GFAP, BLBP, and Nestin.

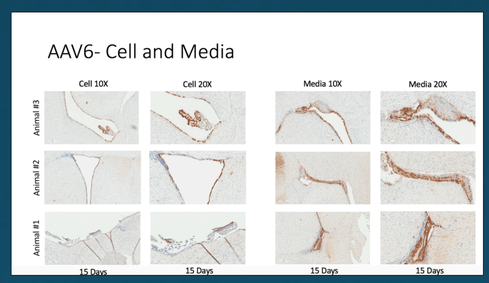


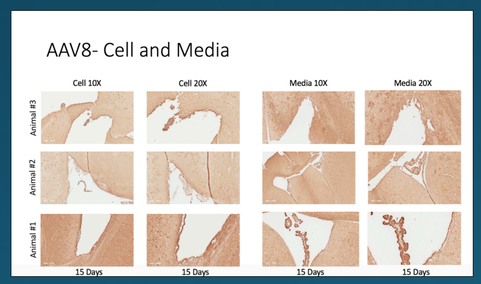
rAAV intraventricular injection into a neonatal mouse (p0)

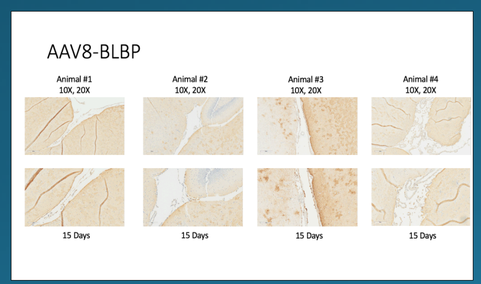


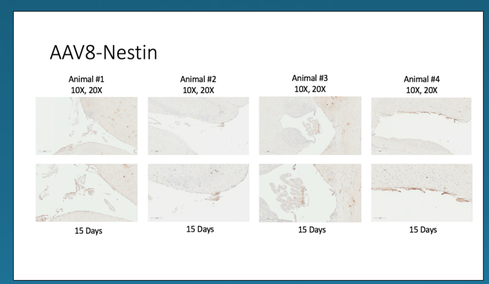












Results

rAAV8 with the CBA promoter resulted in the most apparent tropism. Looking at the figures below, the lateral ventricles of the brain tissue that was treated with rAAV8 and the CBA promoter are heavily stained. Thus, they are easily distinguished. There was little to no indication of tropism for the ventricles of the brain tissue that were treated with rAAV8 and the GFAP and Nestin promoters.

Conclusion

After 15 days, we found that rAAV tropism was most apparent with rAAV8 using the CBA promoter. The ventricle's cells were heavily stained and distinguished from the other tissues of the brain. These findings reflect rAAV8's tendency to successfully transduce and predict the expression of EGFP in an animal model. Little to no staining occurred in the ventricle cells that were treated with rAAAV8 using the GFAP, BLBP, and Nestin promoters.

With these results, we can further our investigations of rAAV intraventricular injections into neonatal mouse brains. These investigations will lead to a better understanding of the brain ventricular system, especially the structure and function of the ependymal cells which are responsible for the production of the cerebrospinal fluid. These cells are an attractive target for gene therapy, and provide extensive opportunities for research on neurodegenerative diseases.

Future Work

To validate that rAAV8 is transducing the radial glia, we've decided to continue the study through a colocalization experiment using antibody cell markers. If spatial overlap between two antibody cell markers occurs, then rAAV8 has successfully transduced the radial glia.

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