Inner Ear Tropism of Natural and Engineered AAV Serotypes in Non-Human Primate Enables Therapeutic Targeting of a Diverse Set of Cochlear Cell Types

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1. Introduction

Often, the AAV capsid is a primary determinant of cellular transduction for AAV gene therapy vectors, and different capsids can lead to different efficiency or specificity (tropism) of transduction. Because in some therapeutic contexts, capsid tropism can limit the desired transduction and thus enable the therapeutic approach, much research over the past decade has gone into engineering or evolving capsids with novel tropisms. Another proposed advantage of some engineered capsids is that because of their novel amino acid sequence, they may be less likely to be neutralized by pre-existing antibodies (neutralizing antibodies) present in human populations.

In the ear, several novel capsids have been proposed to provide improved transduction of therapeutically relevant cell types, including, for example, Anc80L65, AAVie, and Php.b. Most of the data supporting the value of these, however, have been generated in mice. We sought here to examine the ability of natural and engineered capsids to transduce cells, particularly inner hair cells, in the non-human primate (NHP).

2. Methods

Non-human primates (NHP) and mice were injected with various AAV serotypes expressing eGFP under ubiquitous promotors. All NHP in this study were injected via the Round Window Membrane and mice through the semicircular canals (IL) (Figure 1)

To assess the importance of neutralizing antibody evasion, we inoculated mice twice with AAV8 delivering HA-tag under a CMV promotor and tested the animals for the presence of neutralizing antibodies before delivering AAV8-CMV-GFP to the ear.

We tested NHP for serum levels of pre-existing antibodies before, during the treatment and after treatment with AAV1 at 4 weeks After Cardiac perfusion temporal bones were harvested and decalcified and subsequently processed for whole mount organ of corti preparations or paraffin sectioning.

AAV tropism was visualized using native GFP signal, IHC or RNAscope.

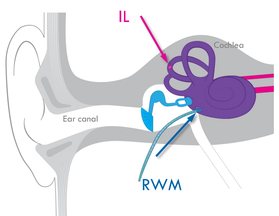

Figure 1: Viral vector administration via the Round Window Membrane and Intra Labyrinth

3. Serotype Comparison in Mice

Figure 2: Cross sections through mouse cochleae showing eGFP in the inner ear after viral transduction with AAV1, AAV8, AAV9 under CAG promotor. AAVs are transducing a variety of cells, but all are showing strong inner hair cell transduction. Scale bars 50 and 500 µm

4. Inner hair cell tropism in Non-Human Primates

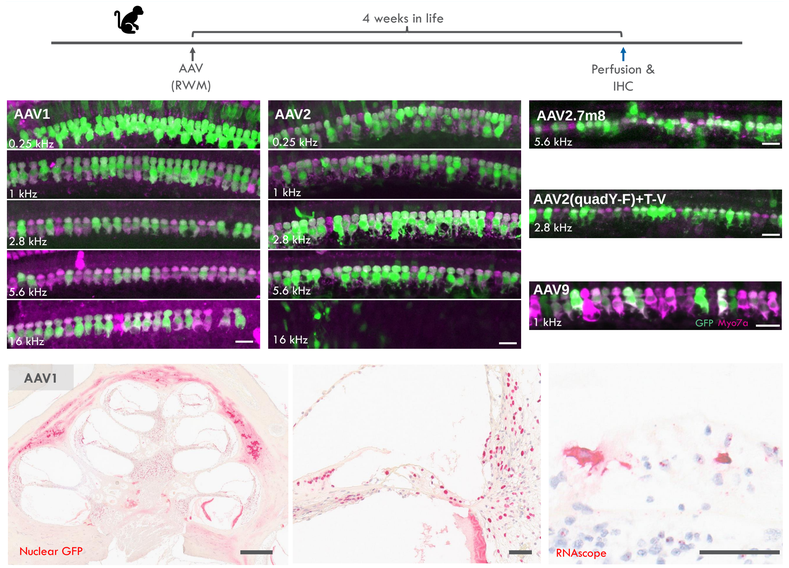

Figure 3 upper: Whole mount organ of corti preparations showing eGFP expression in inner hair cells with a variety of different AAV capsids in *Macaca fascicularis*. Scale bars 20 µm
Lower: AAV1 shows a wide infectivity across all cells in the Cochlea. Scale bars 500 and 50 µm

5. Neutralizing Antibody in Mice

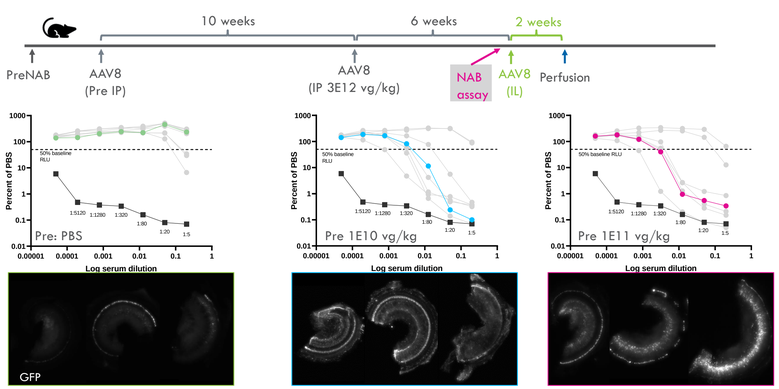

Figure 4: Mice immunized against AAV8 show similar tropism after local (IL) ear delivery of AAV8

5. Neutralizing Antibody in Non-Human Primates

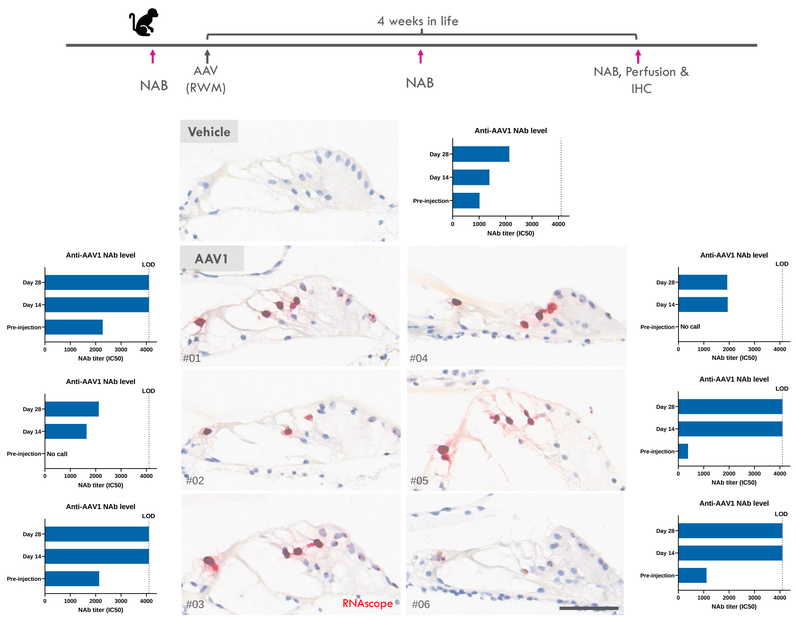

Figure 5: Paraffin sections of AAV1 transduced *Macaca fascicularis* and neutralizing antibody levels. Vector genomes labelled by RNAscope against the promotor. Scale bar 50 µm

6. Summary and Conclusions

Our data suggests that many capsids are viable options for therapeutic development in the inner ear. Especially inner hair cells in the organ of Corti show strong expression with a variety of different vectors in mice and non-human primates.

The existence of pre-existing neutralizing antibodies did not seem to impact the effectivity of transduction, which allows for the use of vectors with high prevalence of seropositivity in the human population.