Poster 905

Combinatorial engineering across multiple surface exposed loops of AAV2 and AAV9 yields capsids with high degrees of enrichment and specificity for target tissues

Xiaojing Shi, Troy Sandberg, Brandon Wheeler, Reem Elteriefi, Michelle Ling, Nicholas Flytzanis, Nick Goeden

Capsida Biotherapeutics, Inc., Thousand Oaks, California, USA

Presented at the American Society of Gene and Cell Therapy (ASGCT) Annual Meeting, 16–19 May 2022, Washington DC, USA

Introduction

Many central nervous system (CNS) indications require broad delivery of genetic therapies throughout the brain and/or spinal cord. Intravenous (IV) delivery of engineered capsids capable of crossing the blood–brain barrier is well-suited to achieving broad and uniform transduction of the CNS. However, there are several ongoing clinical trials focused on CNS or muscle therapeutic areas that have observed severe adverse events related to liver toxicity due to the high viral burden of wildtype adeno-associated virus (AAV) serotypes on the liver.

Here, we employed Capsida Biotherapeutics' combinatorial engineering strategy to evolve novel AAV capsids that are simultaneously enriched in the CNS and de-enriched in the liver following IV administration in non-human primates (NHPs), thereby significantly improving therapeutic potential.

Methods

Library screening study

- Variants were assembled into Capsida's library constructs and viral libraries were produced in HEK293T cells by transfection
- Variant frequency in the viral library was attained by next generation sequencing (NGS) processing of the virus: enzymatic degradation of the capsids followed by polymerase chain reaction (PCR) on the resulting exposed single stranded DNA cargo
- Cynomolgus macaques were injected with 1–2 x 10^{13} vg/kg of viral library intravenously. Two weeks later, tissues were collected and processed for NGS analysis

Pooled variant characterization study

- Tens of capsids were produced separately, packaging a gene of interest and distinct DNA barcodes driven by a ubiquitous promoter, and injected as a pool into three NHPs at a total dose of 1 x 10^{13} vg/kg
- 2 weeks after injection, tissues were collected and processed; DNA and RNA were extracted from tissue samples for NGS analysis

Results

- Screening of the multi-position mutagenesis library in NHPs yielded capsids that are detargeted from the liver while improving on the parent capsid's CNS tropism (Figure 2)
- Combinatorial loop modification of the CNS-tropic capsid, Capsida AAV9 generation 4 vector, generated a liver-detargeting capsid, Capsida generation 4.L-, which showed comparable levels of CNS tropism and 10-fold lower liver enrichment compared with the generation 4 vector in the pooled variant characterization study (Figure 5)
- CNS-tropic AAV2 variants identified from a structure-guided, rationally-designed library show a widespread reduction in liver tropism

References

1. Goertsen, D *et al. Nat Neurosci* 2022;25:106–15
2. Kern A *et al. J Virol* 2003;77:11072–81.

Capsida vector evolution roadmap

Starting Capsid (e.g., AAV9, AAV2)

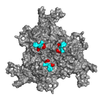
Initial library screening

Variant optimization and re-diversification

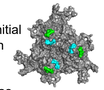
Initial library screening starts with libraries of billions of novel capsids, which are constructed by peptide insertions at surface-exposed variable region (VR)-VIII loops; these are narrowed down to top performing candidates over two rounds of screening in NHPs, rodents, and human cell lines.

Variant optimization and re-diversification libraries are focused on improving tropism for target tissues and detargeting off-target tissues like the liver.

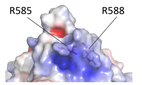
Liver detargeting engineering strategy



Multi-position modification
Variants with tropism toward the target tissue are identified from initial library screening studies, in which peptide insertions were added to surface-exposed VR-VIII loop of wildtype AAV. We re-diversify these variants by introducing random mutations across multiple positions of the inserted peptide loop to detarget off-target tissues.



Combinatorial loop modification
Iterative modifications are made to the two adjacent surface-exposed loops at the three-fold axis of AAV. Mutations at VR-VIII loop first confer a new phenotype, i.e., CNS tropism, and the following loop VR-IV alterations then refine the phenotype away from off-target tissues.[1]



Structure-guided multiple serotype engineering
Positively charged residues in VR-VIII loop of AAV2 are involved in binding of heparan sulfate proteoglycan (HSPG), the primary receptor of AAV2.[2] Peptide modifications in the loop may ablate binding of HSPG, therefore detargeting the liver while screening for novel receptor interactions that enable CNS tropism.

Multi-position modification

Figure 1. Schematic of multi-position modification engineering of the CNS-tropic Capsida AAV9 generation 4 vector

In initial library screening studies, the Capsida AAV9 generation 4 vector with a peptide insertion at VR-VIII loop was demonstrated to be significantly more enriched in the brain than AAV9. Point mutations were randomly introduced to the inserted peptide loop of the AAV9 generation 4 vector, yielding a variant re-diversification library. Library screening, in which CNS and liver enrichments of variants were measured and analyzed, was carried out in NHPs.

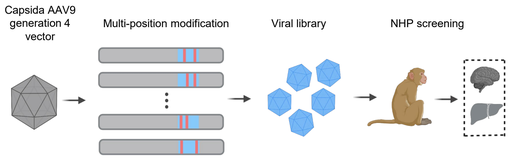
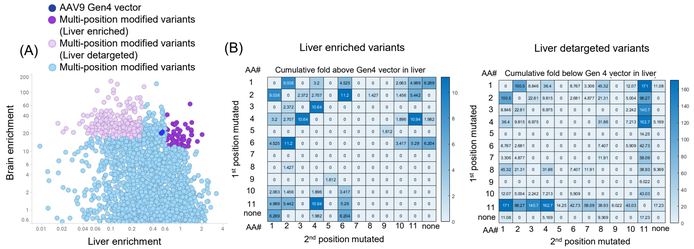


Figure 2. Multi-position mutational modification of Capsida AAV9 generation 4 vector resulted in CNS-tropic capsids that target away from liver

(A) Liver-enriched/detargeted variants with comparable or even higher brain enrichment over the parent capsid were identified from the multi-position modified library. (B) Positional crossplots show different "mutation pair hotspot" patterns of CNS-tropic variants with liver enriching (left) or liver-detargeting (right) properties, indicating positions for additional mutagenesis and distinct molecular mechanisms that can be further explored.



Combinatorial loop modification

Figure 3. Schematic of combinatorial loop modification of the CNS-tropic Capsida AAV9 generation 4 vector

Random amino acid substitutions at VR-IV loop were paired with the Capsida AAV9 generation 4 vector insertional peptide at the VR-VIII loop. Library screening, in which CNS and liver enrichments of variants were measured and analyzed, was carried out in NHPs.

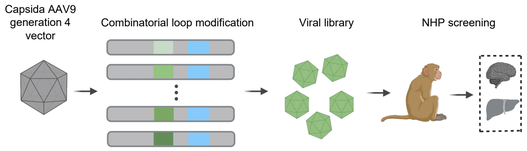
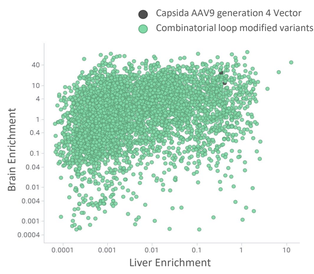
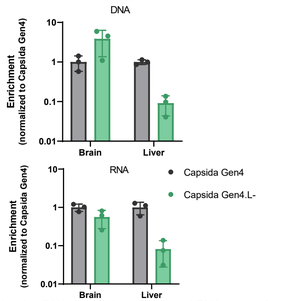


Figure 4. Generation 4 vector with VR-IV modifications populationally detarget from the liver



Top liver detargeted capsids retain the CNS penetrance of the parent AAV9 generation 4, but with greatly reduced liver presence.

Figure 5. Liver-detargeting variant showed comparable CNS penetrance and 10-fold lower liver enrichment than the parent capsid



Graphs showing DNA distribution (upper) and RNA expression (lower) of generation 4 and generation 4.L- capsids in the pooled variant characterization study. Each dot represents individual NHPs in the study.

Structure-guided multiple serotype engineering

Figure 6. Schematic of initial library screening of AAV2 variants

The AAV2-based capsid library was constructed by altering the VR-VIII loop of AAV2 and screened in NHPs for CNS tropism.

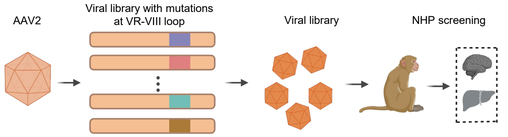
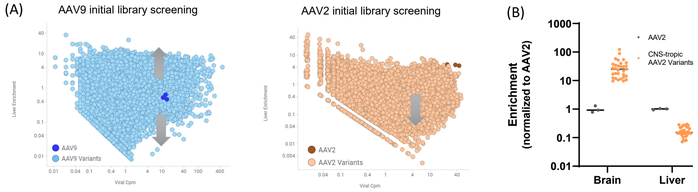


Figure 7. AAV2 variants populationally detarget from liver compared with the parent wildtype AAV2 capsid



(A) Figures present the variants' distribution of liver enrichment in the AAV9 (left) and AAV2 (right) initial library screenings. AAV9 variants showed bidirectional distribution in liver enrichment relative to the parent AAV9. AAV2 variants populationally are less enriched in the liver than AAV2. (B) Variants showing brain tropism were identified from AAV2 initial library screening and exhibited a liver detargeting property.

Conclusions

- Combinatorial engineering across multiple surface exposed loops of multiple serotypes was built into Capsida's vector evolution roadmap
- These results demonstrate that multiple engineering strategies can effectively detarget capsids from off-target tissues, while maintaining tropism to the tissue of interest

Poster PDF

Video

Please scan this quick response (QR) code with your smartphone camera or app to obtain a copy of these materials. Alternatively, please click here to download the video.