Every little bit helps: A single-residue switch in a vascular AAV enables blood-brain barrier penetration

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The clinical potential of adeno-associated virus (AAV) vectors as gene delivery tools for neurological diseases is constrained by the low capacity of natural AAV capsids to cross the blood-brain barrier (BBB). As a result, delivery of AAV vectors to the central nervous system (CNS) requires high vector doses or invasive local delivery, both associated with significant risks of vector-induced toxicity. To overcome this limitation, multiple groups have attempted to generate engineered AAV capsids with improved BBB penetration and CNS transduction.

A previous study from Körbelin et al. performed directed evolution of an AAV2 peptide display library in mouse brain and identified AAV2-BR1, a capsid showing strong and highly specific transduction of the brain vasculature.[1] In this issue of *Molecular Therapy – Methods and Clinical Development*, Hayato Kawabata and colleagues build on this body of work and show that a single mutation in the AAV2-BR1 capsid increases BBB penetration and causes a dramatic shift of vector tropism from the vasculature toward neurons.[2] The authors later demonstrate that a similar shift in tropism can be achieved by generating mosaic capsids combining unmodified AAV2-BR1 and AAV9 subunits. These findings have important implications for AAV vector engineering, and they contribute to a better understanding of the molecular mechanisms controlling AAV tropism, endocytosis, vascular transcytosis (i.e., AAV transport from the bloodstream to the surrounding tissue through endothelial cells) and eventually transduction.

The idea of using directed evolution to engineer AAV capsids with enhanced tropism for specific tissues was first proposed in 2003[3,4] and since has led to the discovery of multiple designer capsids with improved properties (reviewed in Becker et al.[5]). This evolution process is generally empirical, relying on the enrichment of certain mutants in cultured cells or animal tissues from large libraries of capsids harboring random mutations, chimeric sequences, or insertions. In most cases, the mechanisms leading to capsid phenotypic changes are yet to be discovered. Recent studies, however, have started to shed light on those molecular mechanisms by identifying receptors used by natural AAV capsids, such as KIAA0319L/AAV receptor (AAVR)[6] or by engineered variants such as the highly neurotrophic capsid PHP.B.[7] In this latter case, the receptor was identified as Ly6A/SCA-1, a protein expressed on the surface of endothelial cells and essential for transcytosis of the capsid across the BBB. The receptor used by AAV2-BR1 to target the brain vasculature remains unknown, but the results obtained by Kawabata et al. provide important insight into the mechanisms of receptor-mediated transcytosis and transduction used by AAV capsids.

AAV2-BR1 was isolated from an AAV2 peptide display constructed according to the method published by Müller et al. in 2003.[1,3] The constructs in this library contain a N587Q mutation adjacent to the randomized peptides, initially incorporated to facilitate the insertion of library-encoding oligonucleotide pools. In the present study, Kawabata et al. reverted this mutation to the parental N587 residue to generate the AAV2-BR1N capsid. This single-residue modification caused a profound change in tropism by allowing the capsid to undergo transcytosis across the BBB and transduce cells of the brain parenchyma instead of being "trapped" in the endothelial cells. This transduction-to-transcytosis switch suggests that the N587 residue participates in an essential interaction of the capsid with one or more cellular factors implicated in endosomal trafficking.

Several models could account for these findings. In the first "single receptor" model, the Q587N reversion adjacent to the peptide presented on the AAV2-BR1 surface would modulate the affinity of the ensuing AAV2-BR1N capsid for its endothelial receptor. This change in capsid-receptor interaction could impact the release of the viral capsid from the receptor in the endocytic system, shifting the fate of the capsid from endosomal escape (and endothelial cell transduction) to transcytosis and egress from the basolateral membrane. In an alternative "receptor switch" model, the Q587N reversion would restore the binding of the capsid to a secondary receptor involved in cell-surface attachment and/or endosomal trafficking, such as heparan sulfate proteoglycan (HSPG) or AAVR. In this model, the original AAV2-BR1 capsid would be able to transduce vascular cells via its endothelial receptor but would be defective in extravasation or transduction of non-vascular cells due to impaired interaction with a secondary receptor caused by the N587Q mutation. This loss-of function would be rescued either by Q587N reversion in AAV2-BR1N or by the incorporation of AAV9 capsid subunits in mosaic AAV2-BR1/AAV9 capsids.

The N587 residue lies between two arginines involved in HSPG binding (R585 and R588) but also in the vicinity of Q589, which directly contributes to the interaction with AAVR.[8] Several observations suggest that

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AAVR, rather than HSPG, may be involved in the phenotypic switch observed by Kawabata and colleagues. First, the transcytosis phenotype is rescued by the incorporation of capsid subunits from AAV9, which utilizes galactose and not HSPG as an attachment glycan.[9] Second, N587 mutation has no measurable impact on AAV2 affinity for HSPG but can impair transduction at a post-binding stage,[10] more consistent with the involvement of AAVR. Lastly, the observations from the present study are strikingly reminiscent of findings from the Deverman group with PHP.eB showing that the depletion of AAVR leads to a dramatic shift of tropism from neurons to endothelial cells.[7] It is possible that the results from Deverman and from the present study converge toward the same phenomenon, in which an engineered capsid could utilize its *de novo* vascular receptor to transduce endothelial cells but would be defective in transcytosis and/or neuronal transduction when capsid-AAVR interaction is impaired. A comparison of AAV2-BR1 and AAV2-BR1N affinity for AAVR could provide further support to the receptor switch hypothesis.

AAV2-BR1N did not show improved brain transduction in a primate model, which questions the clinical relevance of the findings. Nevertheless, this study represents an important step in the understanding of the complex, multistep binding-transcytosis-transduction process that is required for efficient brain transduction with AAV via intravenous dosing. The present study examined the impact of a single point mutation, but there is no doubt that the vast sequence space of AAV holds many more surprises and opportunities that could be harnessed for iterative vector improvement. The findings of Kawabata et al. have pivotal implications for the engineering of novel capsids, the understanding of combined action of multiple receptors, and eventually the semi-rational engineering of second- or third-generation variants with enhanced properties.

DECLARATION OF INTERESTS
The authors declare no financial interest.

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