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Taking a Hint from Structural Biology: To Better Understand AAV Transport across the BBB

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The blood-brain barrier (BBB) poses a significant challenge to therapy development for diseases afflicting the CNS because it prevents most drugs from entering the parenchymal of brain and spinal cord when delivered systemically. Surprisingly, some recombinant adeno-associated viruses (rAAVs) packaged into certain serotype capsids, such as AAV9 and AAVrh.10, can cross the BBB and deliver gene therapy into neurons and astrocytes spread across the CNS.[1–4] Although systemic rAAV has been tested to treat CNS diseases in various animal models and in clinical trials, the mechanism by which it crosses the BBB remains unknown. In this issue of *Molecular Therapy*, Albright and colleagues[5] report a footprint of eight residues from the AAVrh.10 capsid that contributes to its ability to cross the BBB. This study reveals new insight into transendothelial AAV transport at the BBB and provides a roadmap for engineering AAV capsids for CNS gene therapy.

As a gene delivery vehicle, rAAV is composed of a recombinant DNA genome carrying a transgene and a non-enveloped protein capsid derived from a diverse pool of naturally existing AAV capsid genes. Successful gene delivery and transgene expression involves a series of cellular events, including receptor and co-receptor binding, endocytosis, intracellular trafficking, endosomal escape, nuclear entry,

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capsid uncoating, genome release and processing, and transgene transcription and translation.[6] Most of these processes are determined by the rAAV capsid and its interaction with host factors. The capsid also shapes other clinically relevant features of rAAV when used for gene therapy, such as the likelihood of being neutralized by pre-existing AAV capsid antibodies in human populations.[7] Therefore, extensive research efforts have been focused on elucidating the structural determinants of various AAV capsids that are required for specific tissue tropism and immunogenicity.[8,9] Furthermore, these insights into the structural biology of natural AAV capsids can facilitate capsid engineering to develop safer and more efficacious gene therapy vectors.[10,11]

The groundbreaking discovery that certain AAV serotype capsids can cross the BBB opened a new avenue for treating widespread CNS pathologies via a convenient intravenous injection.[1–4] However, what capsid features distinguish these particular AAV serotypes from the rest regarding the CNS phenotype remains a puzzle. To investigate this issue, Albright et al.[5] first generated a library of chimeric capsid variants through DNA shuffling using AAV1 and AAVrh.10 as the parental capsids. These two capsids share 85% amino acid sequence homology but display distinct CNS phenotypes: AAV1 transduces only brain vasculature, whereas AAVrh.10 can cross the BBB and transduce neurons and astrocytes in addition to blood vessels. This AAV1/rh.10 domain swap library comprising 36 clones enabled a comparative study to examine the correlation between capsid sequence and the capability of crossing the BBB.

The authors selected six chimeric variants from the library for *in vivo* characterization, considering packaging efficiency and structural analysis. They packaged a GFP-expressing cassette into AAV1, AAVrh.10, or the six chimeric variants and delivered individual vectors into adult mice via tail vein injection at a dose of 5×10^{11} viral genomes (vg) per mouse. As expected, immunostaining of brain sections showed that AAV1 vector transduces only vasculature and AAVrh.10 transduces neurons, glia, and endothelial cells. Interestingly, two chimeric variants, AAV1R6 and AAV1R7, demonstrated robust transduction of cortical neurons but reduced transduction of glia and vasculature relative to the parental AAVrh.10. The authors carefully examined the transduction profile in various brain regions, including cerebral cortex, hippocampus, thalamus, hypothalamus, striatum, and amygdala. Overall, they found that AAV1R6 and AAV1R7 transduce neurons similarly as AAVrh.10 but display reduced glial transduction and diminished transduction of endothelial cells of vasculature. Furthermore, both chimeric variants are detargeted from the liver relative to their parental capsids while retaining the cardiac transduction profile of AAV1 and AAVrh.10.

Although AAV1R6 and AAV1R7 are 97%–98% identical to AAV1 in amino acid sequence, the ability to cross the BBB and transduce neurons is reminiscent of AAVrh.10. Therefore, these chimeric variants provide a unique opportunity to evaluate a handful of AAVrh.10-derived residues for their contribution to crossing the BBB. To narrow the list of residues for further study, the authors carried out structural analysis of the 22 AAVrh.10-derived residues in AAV1R6 and AAV1R7. They excluded residues that are not exposed on the capsid surface as well as those located in regions important for viral genome packaging and release. They focused on a stretch of eight surface-exposed amino acids within a region previously shown to be important in determining the tropism and antigenicity of AAV1. Indeed, when they grafted the eight amino acids derived from AAVrh.10 onto AAV1, this minimal footprint enabled the engineered chimeric capsid, designated as AAV1RX, to cross the BBB and selectively transduce neurons. Therefore, the 8-residue footprint is critical for crossing the BBB. The authors also noticed that the AAVrh.10-derived footprint is highly conserved in other neurotropic vectors, further suggesting that this footprint is critical for crossing the BBB.

It is important to note that the study was performed in the context of AAV1 and AAVrh.10. For example, other residues in the AAV1 capsid may play a synergistic role with the AAVrh.10-derived footprint to confer a CNS phenotype. Whether the footprint is necessary or sufficient to enable the other serotype capsids to cross the BBB remains unknown. Nevertheless, the identification of the minimal footprint is an important step toward understanding the mechanism underlying AAV transport across the BBB from a structural biology perspective. To fully understand the precise mechanism, host factors must also be considered, and other experimental approaches will be instrumental. For instance, an *in vitro* BBB model consisting of primary human brain microvascular endothelial cells has been used to study the trafficking of AAV vectors across the BBB.[12] This *in vitro* BBB model revealed insights into the interaction between rAAV and BBB in the process of transendothelial movement, suggesting that the transport of AAV9 vector across the BBB is an active, cell-mediated process, and that it does not disrupt the BBB integrity.[12]

The new study by Albright et al.[5] is also important for expanding the vector tool box for CNS gene therapy. The engineered AAVR1X capsid can not only cross the BBB and transduce neurons, but also shows markedly reduced transduction in the liver and, therefore, may have a better safety profile. The 8-residue footprint identified in this study may also guide future capsid engineering to improve CNS gene delivery. For vector development, directed evolution and library selection is another approach to engineering novel AAV capsid that can cross the BBB without prior knowledge about capsid structure and function.[13] Importantly, the capsids yielded from these studies have to prove their efficacy in large animals, such as non-human primates, before their successful implementation in clinical use.

The capsid footprint identified in this study provides a clue about the structural biology that enables some AAV serotype capsids to cross the BBB. The footprint may serve as the starting point to unfold the detailed transendothelial events at the BBB, which should involve an array of interactions among AAV vectors, the host cells, and the

extracellular environment. With the guidance of structural biology, as demonstrated in this new study, rational engineering promises to further refine AAV capsid for safer and more efficacious gene therapy vectors.

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Intraoperative Molecular Imaging in Lung Cancer: The State of the Art and the Future

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Lung cancer is the deadliest malignant disease in the United States and has a median survival of only 5–11 months at advanced stages.[1] However, for patients with stage I–IIIA cancers, minimally invasive surgery with curative intent plays a key role in treatment,[2] and improving signal to noise in identifying diseased tissue is therefore critical in guiding resection. Non-targeted dyes, such as fluorescein (FITC) or indocyanine green (ICG), have long been administered pre-operatively to improve tumor contrast and have significantly improved surgical outcomes.[3,4] More recently, targeted probes have been developed that utilize antibodies, aptamers, or peptides to bind to cancerous tissue more specifically and further enhance the tumor-to-background ratio (TBR).[5,6] In this issue of *Molecular Therapy*, Predina et al.[7] report on their pilot clinical trial of a near-infrared small-molecule probe targeting folate receptor-α (FRα) to sensitively detect pulmonary adenocarcinomas (PAs) during surgery. In line with previous clinical trials of FRα-targeted probes in ovarian and colorectal cancers and of fluorescently-labeled anti-EGFR antibodies, such as cetuximab-IRD800, in head and neck cancer, this trial lends further support to the clinical utility of intraoperative molecular imaging (IMI) in identifying lesions that might otherwise be missed.[8–11]

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