Establishment of a Predictive Transcytosis Model to Recapitulate Capsid-Receptor Interaction and Phenotype of BBB-penetrant AAV Variant

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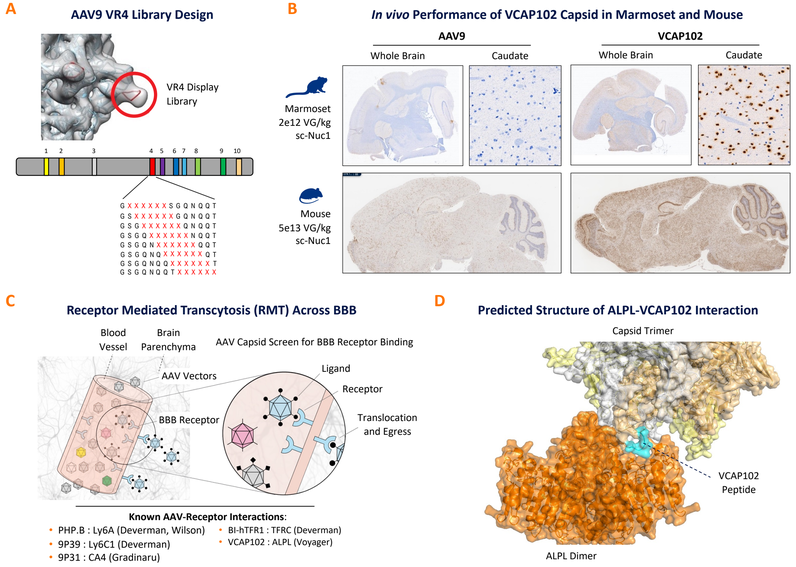
INTRODUCTION

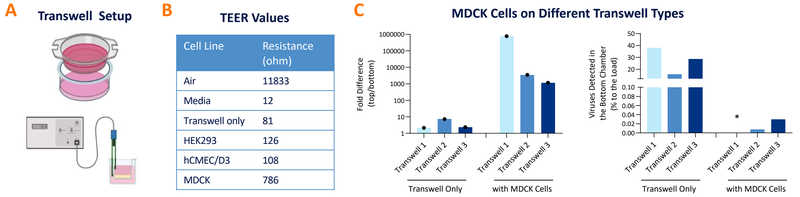
Delivery of genetic payloads across the blood-brain barrier (BBB) remains a critical challenge for treatment of neurological diseases. Recent years have seen rapid progress in the engineering of BBB-penetrant viral and non-viral gene transfer vehicles, but questions remain regarding the translatability of these discoveries to human patients. In the absence of true in-human observations, translation between animal models and humans can be inferred from phenotypic equivalence across various primate and/or rodent species, or from the identification of the molecular mechanisms of BBB penetration, for example by identification of a vascular receptor. We have previously identified VCAP-102, an Adeno-Associated Virus (AAV) capsid variant showing improved BBB penetration in both primates (old world and new world) and rodents, and we were able to determine that VCAP-102 binds to a BBB surface receptor showing a high degree of conservation across species, including humans. In order to further characterize the mechanism of action of VCAP-102, we generated a transwell model using Madin-Darby Canine Kidney (MDCK) cells overexpressing VCAP-102 receptor and showed that this cell line allowed very efficient transcytosis of VCAP-102 capsids, but not the parental AAV9. Conversely, parental MDCK cells, which do not naturally express the receptor, were non-permissive to either capsids. We confirmed the receptor-mediated transcytosis of generation 2 capsids in our transwell model, consistent with their *in vivo* potency.

Figure 1. Discovery of VCAP102 and Identification of ALPL as BBB Receptor



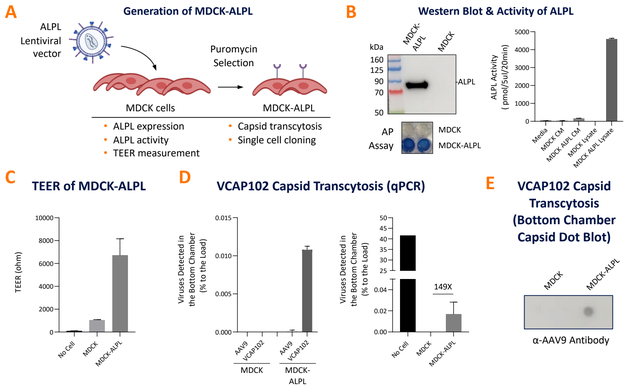
Discovery of ALPL as the primary cell surface receptor responsible for transport of VCAP102 capsid across the BBB. (**A**) TRACER library design and 3D structure of VCAP102 capsid obtained by cryo-electron microscopy. The region representing the 6-AA peptide inserted in AAV9 VR4 loop is highlighted in red. (**B**) Brain transduction by AAV9 (left panels) and VCAP102 (right panels) in marmoset (top panels) and mouse (bottom panels). (**C**) Model for BBB transport of VCAP102 and other neurotrophic capsids, explained by a single capsid-receptor interaction on the lumen of the BBB endothelial cells. Known vascular receptors responsible for increased brain transduction by evolved AAV capsids are listed on the bottom. Of note, Ly6A and Ly6C1 are mouse-specific genes that do not have an ortholog in primates. Ly6A, Ly6C1, CA4 and ALPL belong to the GPI-AP family of membrane-associated proteins. (**D**) *In silico* model of the interaction of VCAP102 capsid with ALPL, predicted by AlphaFold. ALPL dimer is represented in orange, the three capsid subunits forming the threefold axis protrusion of VCAP102 are depicted in white, wheat and yellow. The 6 residues specific for VCAP102 are colored in cyan.

Figure 2. MDCK Cells Show Tight Barrier Properties in a Transwell Model



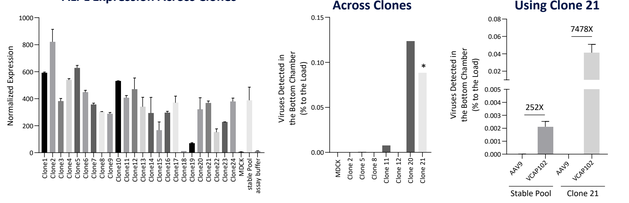
Establishment of transwell model for transcytosis and selection of transwell types. (**A**) Schematic showing top and bottom chamber separated by transwell insert. ERS-2 voltohmmeter to measure Trans Epithelial Electrical Resistance (TEER). (**B**) Typical TEER readings for different cell lines. (**C**) Evaluating different transwell types for barrier formation by Madin Darby Canine Kidney (MDCK) cells. MDCK cells were allowed to grow on three types of transwell inserts for 2-3 days at 37 °C with 5% CO2 under humidified conditions. Transwells without cells are used as a control. Virus was added to the top chamber of the transwell and after overnight incubation, media from both top and bottom chamber were collected for qPCR-based quantification. The virus in the bottom chamber is expressed as a percentage of total virus input. Small variations in transwell efficiency to form tight barrier were observed and transwell 1(*) was found to be optimal for establishment of model.

Figure 3. VCAP102 Shows Transcytosis in MDCK-ALPL Transwell Model



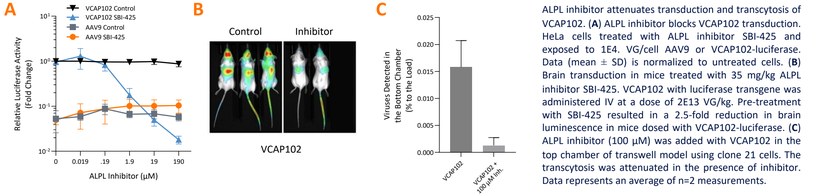
Establishment of stable MDCK cell line expressing ALPL. (**A**) Schematic showing the process of stable pool generation. (**B**) Western blot of the lysate from MDCK or MDCK-ALPL cells probed with an anti-ALPL antibody. ALPL activity is specific MDCK-ALPL cell lysate but not parental MDCK cells. (**C**) ALPL promotes the formation of MDCK barrier. (**D**) qPCR detection of AAV9 or VCAP102 in the transwell model using parental MDCK or MDCK-ALPL cells. VCAP102 in the bottom chamber expressed as a percentage of total virus input shows 149-fold difference between MDCK-ALPL and parental MDCK cells. Value represents mean ± SD (n=3). (**E**) Dot blot to detect virus in the bottom chamber with an anti-AAV9 antibody.

Figure 4. Higher Level of Transcytosis Observed Using Single MDCK-ALPL Clone



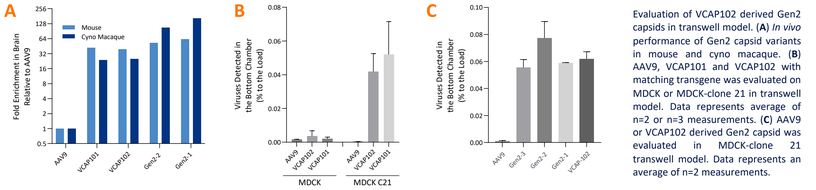
Isolation of clone 21 from MDCK-ALPL stable pool. (**A**) Limited dilution of MDCK-ALPL stable pool was performed to isolate 24 clones. ALPL expression across clones was measured and normalized against total protein concentration. (**B**) Selected clones were tested in the transwell model using VCAP102. Clones 20 and 21(*) showed the highest level of transcytosis. (**C**) AAV9 or VCAP102 with matching transgene was tested in transwell model using clone 21 or stable pool. Clone 21 shows higher transcytosis compared to polyclonal MDCK-ALPL pool.

Figure 5. ALPL Inhibitor Blocks VCAP102 Transcytosis



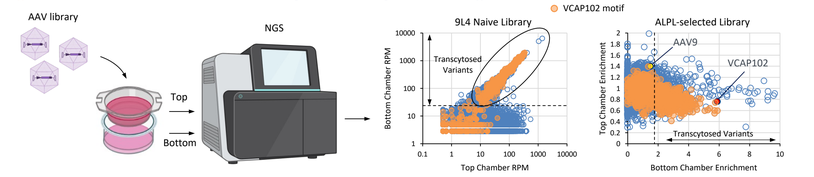
ALPL inhibitor attenuates transduction and transcytosis of VCAP102. (**A**) ALPL inhibitor blocks VCAP102 transduction. HeLa cells treated with ALPL inhibitor SBI-425 and exposed to 1E4. VG/cell AAV9 or VCAP102-luciferase. Data (mean ± SD) is normalized to untreated cells. (**B**) Brain transduction in mice treated with 35 mg/kg ALPL inhibitor SBI-425. VCAP102 with luciferase transgene was administered IV at a dose of 2E13 VG/kg. Pre-treatment with SBI-425 resulted in a 2.5-fold reduction in brain luminescence in mice dosed with VCAP102-luciferase. (**C**) ALPL inhibitor (100 µM) was added with VCAP102 in the top chamber of transwell model using clone 21 cells. The transcytosis was attenuated in the presence of inhibitor. Data represents an average of n=2 measurements.

Figure 6. Gen2 and Other Related Capsids Show Similar Extent of Transcytosis as VCAP102



Evaluation of VCAP102 derived Gen2 capsids in transwell model. (**A**) *In vivo* performance of Gen2 capsid variants in mouse and cyno macaque. (**B**) AAV9, VCAP101 and VCAP102 with matching transgene was evaluated on MDCK or MDCK-clone 21 in transwell model. Data represents average of n=2 or n=3 measurements. (**C**) AAV9 or VCAP102 derived Gen2 capsid was evaluated in MDCK-clone 21 transwell model. Data represents an average of n=2 measurements.

Figure 7. Use of MDCK-ALPL as Capsid Library Screening Tool



Use of MDCK-ALPL as a capsid library screening tool. A random peptide display AAV9-VR4 library or a synthetic library pre-selected for ALPL binding were added to the top chamber of MDCK-ALPL cells plated in a transwell. Virus was collected from the top and bottom chambers and viral DNA was amplified by PCR and subjected to NGS sequencing. Left panel: data obtained with random library, dots indicates reads per million (RPM) of each variant in the top and bottom chamber, capsid variants containing the consensus VCAP-102 motif are highlighted in orange. Right panel: data obtained with the synthetic ALPL-selected library. Values indicate the RPM of each variant normalized to the input viral library (enrichment score). AAV9 and VCAP-101 controls are indicated.

CONCLUSIONS

- Tissue-Nonspecific Alkaline Phosphatase (ALPL) was identified as the receptor responsible for Receptor Mediated Transport (RMT) of VCAP102 across BBB.
- A transwell model using MDCK cells showed low paracellular transport and high TEER value. VCAP102 showed transcytosis in MDCK-ALPL model, while parental AAV9 did not. A single clone of MDCK-ALPL showed better transcytosis than stable pool.
- ALPL inhibitor blocked transcytosis of VCAP102.
- Transcytosis property was also observed in other VCAP102 derived Gen2 capsids.
- MDCK transwell model recapitulates ALPL-mediated property of BBB-penetrant capsid and could be used as an efficient tool for capsid library screening.