

doses should result in better therapeutic efficacy while lowering safety issues, such as immunotoxicity.

SUMMARY

Described herein are methods that use an adeno-associated virus (AAV) vector genome with a two-part expression cassette to identify novel virus clones. The first is a Cre-recombinase cassette under a promoter of interest. The second part is an AAV promoter to drive expression of an engineered capsid gene, cloned “in cis” to the first section of the viral genome. Virus vectors are selected for transgene expression (highly sensitive Cre expression) using cells that express a reporter gene (e.g., green fluorescent protein) with an upstream loxP/stop site, thus preventing reporter expression until AAV vector-delivered Cre removes the stop site. Reporter gene positive cells can be isolated and recovered AAV capsid sequences will have a higher likelihood of mediating efficient transgene expression. Also described herein are engineered viral sequences that drive efficient expression in the central nervous system (CNS) and peripheral nervous system (PNS), heart, liver, and inner ear.

Thus, provided herein are AAV capsid proteins comprising an amino acid sequence that comprises at least four contiguous amino acids from the sequence STTLYSP (SEQ ID NO:1) or FVVGQSY (SEQ ID NO:2). In some examples, the AAV capsid proteins comprise an amino acid sequence that comprises at least five contiguous amino acids from the sequence STTLYSP (SEQ ID NO:1) or FVVGQSY (SEQ ID NO:2). In some examples, the AAV capsid proteins comprise an amino acid sequence that comprises at least six contiguous amino acids from the sequence STTLYSP (SEQ ID NO:1) or FVVGQSY (SEQ ID NO:2). Alternatively, the AAV capsid proteins comprise an amino acid sequence that comprises at least four, five, or six contiguous amino acids from the sequences shown in FIGs. 2A or 7C (SEQ ID NOs:17-150).

In some examples, the AAV is AAV9.

In some examples, the AAV capsid proteins comprises AAV9 VP1.

In some examples, the sequence is inserted into the capsid at a position corresponding to amino acids 588 and 589 of SEQ ID NO:6, at the VP1/VP2 interface (amino acid 138) or any site between 583-590.

Also provided herein are nucleic acids encoding an AAV capsid protein as described herein.

Additionally, provided herein are AAVs comprising the capsid proteins described herein, and preferably not comprising a wild type VP1, VP2, or VP3 capsid protein. In some examples, the AAVs further comprising a transgene, preferably a therapeutic transgene.

Further provided are methods of delivering a transgene to a cell, e.g., a cell in vivo or ex vivo/in vitro. The methods include contacting the cell with an AAV as described herein. In some examples, the cell is a neuron (optionally a dorsal root ganglion neuron or spiral ganglion neuron), astrocyte, cardiomyocyte, or myocyte, astrocyte, glial cell, inner hair cell, outer hair cell, supporting cell, fibrocyte of the inner ear, photoreceptors, interneurons, retinal ganglion, or retinal pigment epithelium.

In some examples, the cell is in a living subject, e.g., a mammalian subject, preferably a human. In some examples, the cell is in a tissue selected from the brain, spinal cord, dorsal root ganglion, heart, inner ear, eye, or muscle, and a combination thereof. In some examples, the subject has Alzheimer's Disease; Parkinson's Disease; X-linked Adrenoleukodystrophy; Canavan's ; Niemann Pick; Spinal muscular atrophy; Huntington's Disease; Connexin-26; Usher Type 3A; Usher Type 2D; Hair cell-related hearing loss; Hair cell-related hearing loss (DFNB7/11); Inner hair cell-related hearing loss (DFNB9); Usher Type 1F; Usher Type 1B; Retinitis pigmentosa (RP; non-syndromic); Leber congenital amaurosis; Leber Hereditary Optic Neuropathy; Usher Syndrome (RP; syndromic with deafness); Duchenne Muscular Dystrophy; Allograft vasculopathy; or Hemophilia A and B. The methods and compositions described herein can be used to treat these conditions, by administration of a therapeutically effective amount of an AAV carrying a therapeutic transgene, sufficient to ameliorate, reduce risk of, or delay onset of one or more symptoms of the condition.

In some examples, the cell is in the brain of the subject, and the AAV is administered by parenteral delivery; intracerebral; or intrathecal delivery.

In some examples, the intrathecal delivery is via lumbar injection, cisternal magna injection, or intraparenchymal injection.

In some examples, the AAV is delivered by parenteral delivery, preferably via intravenous, intraarterial, subcutaneous, intraperitoneal, or intramuscular delivery.

In some examples, the cell is in the eye of the subject, and the AAV is administered by subretinal or intravireal injection.

5 In some examples, the cell is in the inner ear of the subject, and the AAV is administered to the cochlea through application over or through the round window membrane, through a surgically drilled cochleostomy adjacent to the round window, a fenestra in the bony oval window, or a semicircular canal.

Also provided herein are library construct AAVs comprising:

- 10 (i) a sequence encoding a Cre recombinase driven by a promoter;
(ii) a sequence encoding an AAV9 capsid protein with a peptide as described herein, e.g., a heptamer peptide, inserted between the sequences encoding amino acids (aa) 588-589 of the capsid, driven by a promoter, downstream of the Cre cassette. In some examples, the peptide comprises a random peptide sequence or a pre-selected peptide sequence.

15 Further provided are libraries comprising a plurality of the library constructs as described herein. In some examples, wherein the peptide sequences are random, the library comprises library constructs having sequences encoding all possible variants of the heptamer.

Additional provided herein are methods for identifying an engineered capsid that
20 mediates transgene expression in a pre-selected cell type. The methods include: (a) administering the library of claims 23 or 24 to a non-human model animal, preferably a mammal, wherein the cells of the model animal express a loxP-flanked STOP cassette upstream of a reporter sequence; (b) isolating cells of the pre-selected cell type; (c) selecting cells in which the reporter sequence is expressed; (d) isolating at least part of the library construct, preferably a part comprising the
25 heptamer, from the selected cells in which the reporter sequence is expressed from step (c); and (e) determining identity of the heptamers in the library constructs isolated in step (d), wherein the heptamers that are isolated can mediate transgene expression in the pre-selected cell type.

In some examples, the reporter sequence encodes a fluorescent reporter protein.

In some examples, the model animal is transgenic for the loxP-flanked STOP cassette upstream of a reporter sequence, or wherein the loxP-flanked STOP cassette upstream of a reporter sequence can be expressed from a second construct.

In some examples, determining identity of the heptamers in the library constructs comprises using DNA sequencing analysis.

In some examples, the methods also include before and/or after step (e): using PCR to amplify sequences comprising the heptamer sequences, optionally comprising full capsid sequences, from the library constructs isolated in step (d); cloning the amplified sequences back to a second set of library vectors; repackaging the second set of library vectors; and performing steps (a)-(d) or (a)-(e) on the second set of library vectors.

In one aspect, the present disclosure provides an adeno-associated virus (AAV) capsid protein comprising an amino acid sequence that comprises the four contiguous amino acids STTL, wherein the amino acid comprises the sequence STTLYS.

In another aspect, the present disclosure provides a nucleic acid encoding the AAV capsid protein as defined herein.

In another aspect, the present disclosure provides an AAV comprising the capsid protein as defined herein.

In another aspect, the present disclosure provides an ex vivo or in vitro method of delivering a transgene to a cell, the method comprising contacting the cell with the AAV as defined herein.

In another aspect, the present disclosure provides use of the AAV as defined herein in the manufacture of a medicament for the treatment of any one of : Alzheimer's Disease; Parkinson's Disease; X-linked Adrenoleukodystrophy; Canavan disease; Niemann Pick; Spinal muscular atrophy; Huntington's Disease; Connexin-26; Usher Type 3A; Usher Type 2D; Hair cell-related hearing loss; Hair cell-related hearing loss (DFNB7/11); Inner hair cell-related hearing loss (DFNB9); Usher Type 1F; Usher Type 1B; Retinitis pigmentosa (RP; non-syndromic); Leber congenital amaurosis; Leber Hereditary Optic Neuropathy; Usher Syndrome (RP; syndromic with deafness); Duchenne Muscular Dystrophy; Allograft vasculopathy; and Hemophilia A or B in a

subject, wherein the AAV is to be administered to a cell of the subject, wherein the sequence is STTLYSP (SEQ ID NO:1) and is inserted in a position corresponding to amino acids 588 and 589 of SEQ ID NO:6.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and Figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIGs. 1A-B. iTransduce library for selection of novel AAV capsids capable of efficient transgene expression in target tissue. a. Two-component system of the library construct. 1. Cre recombinase is driven by a minimal chicken beta actin (CBA) promoter. 2. p41 promoter driven AAV9 capsid with random heptamer peptide inserted between aa 588-589, cloned downstream of the Cre cassette. **b.** Selection strategy. i. The iTransduce library comprised of different peptide inserts expressed on the capsid (represented by different colors), are injected i.v. into Ai9 transgenic mouse with a loxP-flanked STOP cassette upstream of the tdTomato reporter gene, inserted into the Gt(ROSA)26Sor locus.

CLAIMS

1. An adeno-associated virus (AAV) capsid protein comprising an amino acid sequence that comprises the four contiguous amino acids STTL, wherein the amino acid comprises the sequence STTLYS.
2. The AAV capsid protein of claim 1, wherein the sequence is STTLYSP (SEQ ID NO:1).
3. The AAV capsid protein of claim 1 or 2, wherein the AAV is AAV9.
4. The AAV capsid protein of any one of claims 1-3, comprising AAV9 VP1.
5. The AAV capsid protein of claim 4, wherein the sequence is inserted in a position corresponding to amino acids 588 and 589 of SEQ ID NO:6.
6. A nucleic acid encoding the AAV capsid protein of any one of claims 1-5.
7. An AAV comprising the capsid protein of any one of claims 1-5.
8. The AAV of claim 7, wherein the capsid protein does not comprise a wild type VP1, VP2, or VP3 capsid protein.
9. The AAV of claim 7, further comprising a transgene.
10. The AAV of claim 9, wherein the transgene is a therapeutic transgene.
11. A ex vivo or in vitro method of delivering a transgene to a cell, the method comprising contacting the cell with the AAV of any one of claims 7-10.
12. Use of the AAV of claim 10 in the manufacture of a medicament for the treatment of any one of : Alzheimer's Disease; Parkinson's Disease; X-linked Adrenoleukodystrophy; Canavan disease; Niemann Pick; Spinal muscular atrophy; Huntington's Disease; Connexin-26; Usher Type 3A; Usher Type 2D; Hair cell-related hearing loss; Hair cell-related hearing loss (DFNB7/11); Inner hair cell-related hearing loss (DFNB9); Usher Type 1F; Usher Type 1B; Retinitis pigmentosa (RP;

non-syndromic); Leber congenital amaurosis; Leber Hereditary Optic Neuropathy; Usher Syndrome (RP; syndromic with deafness); Duchenne Muscular Dystrophy; Allograft vasculopathy; and Hemophilia A or B in a subject, wherein the AAV is to be administered to a cell of the subject, wherein the sequence is STTLYSP (SEQ ID NO:1) and is inserted in a position corresponding to amino acids 588 and 589 of SEQ ID NO:6.

13. The use of claim 12, wherein the cell is a neuron (optionally a dorsal root ganglion neuron or spiral ganglion neuron), astrocyte, cardiomyocyte, myocyte, glial cell, inner hair cell, outer hair cell, supporting cell, fibrocyte of the inner ear, photoreceptors, interneurons, retinal ganglion, or retinal pigment epithelium.
14. The use of claim 12 or 13, wherein the cell is in a living subject.
15. The use of any one of claims 12-14, wherein the cell is a mammalian cell.
16. The use of any one of claims 12-15, wherein the cell is in a tissue selected from the brain, spinal cord, dorsal root ganglion, heart, inner ear, eye, or muscle, and a combination thereof.
17. The use of any one of claims 12-16, wherein the cell is in the brain of the subject, and the AAV is to be administered by parenteral delivery; intracerebral; or intrathecal delivery.
18. The use of claim 17, wherein the intrathecal delivery is via lumbar injection, cisternal magna injection, or intraparenchymal injection.
19. The use of claim 17, wherein the parenteral delivery is via intravenous, intraarterial, subcutaneous, intraperitoneal, or intramuscular delivery.

20. The use of any one of claims 12-16, wherein the cell is in the eye of the subject, and the AAV is to be administered by subretinal or intravitreal injection.
21. The use of any one of claims 12-16, wherein the cell is in the inner ear of the subject, and the AAV is to be administered to the cochlea through application over or through the round window membrane, through a surgically drilled cochleostomy adjacent to the round window, a fenestra in the bony oval window, or a semicircular canal.