

Literature

[0005] U.S. Patent Publication No. 2005/0053922; U.S. Patent Publication No. 2009/0202490; Allocca et al. (2007) *J. Virol.* 81:11372; Boucas et al. (2009) *J. Gene Med.* 11:1103.

SUMMARY

[0006] The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to wild-type AAV. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

[0006a] In one aspect, the present disclosure provides for use of a recombinant adeno-associated virus (rAAV) virion, or pharmaceutical composition comprising said virion, in the manufacture of a medicament for treating an ocular disease in an individual in need thereof, wherein the composition comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, wherein the insertion comprises an amino acid sequence selected from LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDTDTTTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product;

wherein the variant capsid protein infects a retinal cell.

[0006b] In one aspect, the present disclosure provides for a recombinant adeno-associated virus (rAAV) virion comprising:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, wherein the insertion comprises an amino acid sequence selected from LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDSTDTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64), and wherein the variant capsid protein confers infectivity of a retinal cell; and
 b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product.

[0006c] In one aspect, the present disclosure provides for a pharmaceutical composition comprising:

- a) any one of the recombinant adeno-associated virus virions as described herein; and
- b) a pharmaceutically acceptable excipient.

[0006d] In one aspect, the present disclosure provides for an isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein is any one of variant AAV capsid proteins as described herein.

[0006e] In one aspect, the present disclosure provides for an isolated, genetically modified host cell comprising the nucleic acid as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figure 1 provides a representative three-dimensional model of AAV2 containing a random heptamer following amino acid 587.

[0008] Figure 2 depicts greater levels of intravitreal transduction by AAV2 7M8 variant (right), relative to AAV2 (left).

[0009] Figure 3 provides representative fluorescence images of retinal cryoslices showing green fluorescent protein (GFP) expression resulting from 7M8 carrying the GFP gene under the control of the ubiquitous CAG promoter (left) or a photoreceptor-specific Rho promoter (right).

[0010] Figure 4 depicts GFP⁺ photoreceptor cells per million retinal cells as counted by flow cytometry, following transduction by 7M8 or by 7M8 bearing 4 tyrosine mutations (7m8.4YF).

[0011] Figure 5 provides an amino acid sequence of AAV2 VP1 (SEQ ID NO:1).

[0012] Figure 6 provides amino acid sequences corresponding to amino acids 570-610 of AAV2 (Figure 5) of AAV capsid protein VP1 of various AAV serotypes.

[0013] Figures 7A-I depict structural improvements in the *Rs1h*^{-/-} mouse retina after gene transfer.

[0014] Figures 8A-D depict functional rescue of the electroretinogram A and B waves following RS1 gene delivery.

[0015] Figures 9A-E depict sustained improvements in retinal thickness measured at 10 months post 7m8-rho-RS1 treatment.

CLAIMS

What is claimed is:

1. Use of a recombinant adeno-associated virus (rAAV) virion, or pharmaceutical composition comprising said virion, in the manufacture of a medicament for treating an ocular disease in an individual in need thereof, wherein the composition comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, wherein the insertion comprises an amino acid sequence selected from LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDSTDTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product;

wherein the variant capsid protein infects a retinal cell.

2. The use according to claim 1, wherein the insertion site is within amino acids 570-611 of the AAV2 capsid protein set forth in SEQ ID NO:1, or the corresponding position in the capsid protein of another AAV serotype.

3. The use according to claim 1 or 2, wherein the retinal cell is a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell.

4. The use according to any one of claims 1 to 3, wherein said medicament is to be administered by intraocular injection.

5. The use according to any one of claims 1 to 4, wherein said medicament is to be administered by intravitreal injection.

6. The use according to any one of claims 1 to 5, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.

7. A recombinant adeno-associated virus (rAAV) virion comprising:

- a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, wherein the insertion comprises an amino acid sequence selected from LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDITDTTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDITTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64), and wherein the variant capsid protein confers infectivity of a retinal cell; and
- b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product.

8. The rAAV virion of claim 7, wherein the insertion site is within amino acids 570-611 of the AAV2 capsid protein or the corresponding position in the capsid protein of another AAV serotype.

9. The rAAV virion of any one of claims 7 to 8, wherein the retinal cell is a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell.

10. The rAAV virion of claim 7, wherein the insertion site is within amino acids 587-588 of the AAV2 capsid protein set forth in SEQ ID NO:1, or the corresponding position in the capsid protein of another AAV serotype.

11. The rAAV virion of any one of claims 7 to 10, wherein gene product is an interfering RNA, an aptamer or a polypeptide.

12. The rAAV virion of claim 11, wherein the polypeptide is selected from the group consisting of: glial derived neurotrophic factor, fibroblast growth factor 2, neurturin, ciliary neurotrophic factor, nerve growth factor, brain derived neurotrophic factor, epidermal growth factor, rhodopsin, X-linked inhibitor of apoptosis, retinoschisin, RPE65, retinitis pigmentosa GTPase-interacting protein-1, peripherin, peripherin-2, a rhodopsin, and Sonic hedgehog.

13. A pharmaceutical composition comprising:

- a) a recombinant adeno-associated virus virion of any one of claims 7 to 12; and
- b) a pharmaceutically acceptable excipient.

14. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein is as defined in any one of claims 7 to 12.

15. An isolated, genetically modified host cell comprising the nucleic acid of claim 14.