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 ~~OF THE INVENTION~~

[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 refers to a pharmaceutical composition comprising a recombinant adeno associated virus (rAAV) virion, for use in the treatment of an ocular disease in a human or non-human primate in need thereof, wherein the composition further comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises: a) an AAV2 capsid protein variant, wherein the AAV2 capsid protein variant comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV2 capsid protein, wherein the insertion comprises an amino acid sequence LALGETTRPA (SEQ ID NO:45); and b) a heterologous nucleic acid comprising a nucleotide sequence encoding an anti-angiogenic polypeptide; wherein said composition is to be administered by intravitreal injection.

[0006b] In another aspect, the present disclosure refers to the 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 a human or non-human primate in need thereof, wherein the composition further comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises: a) an AAV2 capsid protein variant, wherein the AAV2 capsid protein variant comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV2 capsid protein, wherein the insertion comprises an amino acid sequence LALGETTRPA (SEQ ID NO:45); and b) a heterologous nucleic acid comprising a nucleotide sequence encoding an anti-angiogenic polypeptide; wherein said medicament is to be administered by intravitreal injection.

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

~~a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein; and~~

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

~~2. The rAAV virion of claim 1, wherein the insertion is a peptide of Formula I, Formula IIa, Formula III, or Formula IV.~~

~~3. The rAAV virion of claim 2, wherein the insertion comprises an amino acid sequence selected from LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60).~~

~~4. The rAAV virion of claim 1, 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.~~

~~5. The rAAV virion of claim 1, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.~~

~~6. The rAAV virion of claim 1, wherein the rAAV virion exhibits at least 10-fold increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.~~

~~7. The rAAV virion of claim 1, wherein the rAAV virion exhibits at least 50-fold increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.~~

~~8. The rAAV virion of claim 1, wherein gene product is an interfering RNA or an aptamer.~~

~~9. The rAAV virion of claim 1, wherein the gene product is a polypeptide.~~

~~10. The rAAV virion of claim 7, wherein the polypeptide is a neuroprotective polypeptide, an anti-angiogenic polypeptide, or a polypeptide that enhances function of a retinal cell.~~

~~11. A pharmaceutical composition comprising:~~
~~a) a recombinant adeno-associated virus virion of claim 1; and~~
~~b) a pharmaceutically acceptable excipient.~~

~~12. A method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a recombinant adeno-associated virus (rAAV) virion according to claim 1.~~

~~13. The method of claim 11, wherein the gene product is a polypeptide.~~

~~14. The method of claim 11, wherein the gene product is a short interfering RNA or an aptamer.~~

~~15. The method of claim 13, wherein the polypeptide is a neuroprotective factor, an anti-angiogenic polypeptide, an anti-apoptotic factor, or a polypeptide that enhances function of a retinal cell.~~

~~16. The method of claim 13, wherein the polypeptide is 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, or Sonic hedgehog.~~

~~17. A method of treating a retinal disease, the method comprising administering to an individual in need thereof an effective amount of a recombinant adeno-associated virus (rAAV) virion according to claim 1.~~

~~18. The method of claim 17, wherein said administering is by intraocular injection.~~

~~19. The method of claim 17, wherein said administering is by intravitreal injection.~~

~~20. The method of claim 17, wherein the ocular disease is glaucoma, retinitis pigmentosa, macular degeneration, retinoschisis, Leber's Congenital Amaurosis, diabetic retinopathy, achromotopsia, or color blindness.~~

~~21. An isolated nucleic acid comprising a nucleotide sequence that encodes a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the capsid protein GH loop relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein, when present in an AAV virion, provides for increased infectivity of the AAV virion of a retinal cell.~~

~~22. The isolated nucleic acid of claim 21, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589~~

~~and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.~~

~~23. An isolated, genetically modified host cell comprising the nucleic acid of claim 21.~~

~~24. A variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids wherein the amino acid insertion is in the GH loop of a native AAV capsid, wherein the insertion is a peptide of Formula I, Formula IIa, Formula III, or Formula IV.~~

~~25. The variant AAV capsid protein of claim 24, wherein the insertion comprises an amino acid sequence selected from LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60).~~

~~26. The variant AAV capsid protein of claim 24, wherein the insertion site is between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.~~

~~27. A nucleic acid comprising a nucleotide sequence encoding the variant AAV capsid protein of claim 24.~~

1. A pharmaceutical composition comprising a recombinant adeno associated virus (rAAV) virion, for use in the treatment of an ocular disease in a human or non-human primate in need thereof, wherein the composition further comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises:

a) an AAV2 capsid protein variant, wherein the AAV2 capsid protein variant comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental

AAV2 capsid protein, wherein the insertion comprises an amino acid sequence LALGETTRPA (SEQ ID NO:45); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding an anti-angiogenic polypeptide;

wherein said composition is to be administered by intravitreal injection.

2. 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 a human or non-human primate in need thereof, wherein the composition further comprises a pharmaceutically acceptable excipient, and wherein the recombinant adeno-associated virus (rAAV) virion comprises:

a) an AAV2 capsid protein variant, wherein the AAV2 capsid protein variant comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental AAV2 capsid protein, wherein the insertion comprises an amino acid sequence LALGETTRPA (SEQ ID NO:45); and

b) a heterologous nucleic acid comprising a nucleotide sequence encoding an anti-angiogenic polypeptide;

wherein said medicament is to be administered by intravitreal injection.

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

4. The pharmaceutical composition according to claim 1 or the use of claim 2, wherein the insertion site is between amino acids 587-588 of the AAV2 capsid protein set forth in SEQ ID NO:1.

5. The pharmaceutical composition or use according to claim 4, wherein the insertion consists of the amino acid sequence LALGETTRPA (SEQ ID NO:45) such that the AAV2 capsid protein variant comprises the sequence NLALGETTRPAR.

6. The pharmaceutical composition or use of any one of claims 1-5, wherein from about 10^6 to 10^{15} rAAV2 virions are to be administered.

7. The pharmaceutical composition or use of any one of claims 1-5, wherein from about 10^8 to 10^{12} rAAV2 virions are to be administered.

8. The pharmaceutical composition or use of any one of claims 1-7, wherein the anti-angiogenic polypeptide is a soluble vascular endothelial growth factor (VEGF) receptor.

9. The pharmaceutical composition or use of any one of claims 1-7, wherein the anti-angiogenic polypeptide is a VEGF-binding antibody.

10. The pharmaceutical composition or use of any one of claims 1-7, wherein the anti-angiogenic polypeptide is an Fc fusion polypeptide comprising a soluble Flt polypeptide.

11. The pharmaceutical composition or use according to any one of claims 1-10, wherein the ocular disease is exudative age-related macular degeneration.

12. The pharmaceutical composition or use according to any one of claims 1-10, wherein the ocular disease is diabetic retinopathy.

13. The pharmaceutical composition or use according to any one of claims 1-10, wherein the ocular disease is diabetic macular edema.