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 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 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.