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, there is provided a recombinant adeno-associated virus (rAAV) virion comprising:

a) a variant AAV capsid protein, wherein the variant AAV capsid protein comprises a peptide insertion relative to a corresponding parental AAV capsid protein, wherein the insertion site is located between two adjacent amino acids at a position between amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype, wherein the peptide insertion is a peptide of Formula I, Formula IIa, Formula IIb, Formula III, or Formula IV, and wherein the AAV 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, wherein:

i) Formula I is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, and Lys;

X2 is selected from Gly, Glu, Ala, and Asp;

X3 is selected from Glu, Thr, Gly, and Pro;

X4 is selected from Thr, Ile, Gln, and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Arg, Asn, and Thr; and

X7, if present, is selected from Pro and Asn;

ii) Formula IIa is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

each of X1 – X4 is any amino acid;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iii) Formula IIb is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu and Asn;

X2, if present, is selected from Gly and Glu;

X3 is selected from Glu and Thr;

X4 is selected from Thr and Ile;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iv) Formula III is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is Lys;

X2 is selected from Ala and Asp;

X3 is selected from Gly and Pro;

X4 is selected from Gln and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Asn and Thr; and

X7, if present, is Asn; and

v) Formula IV is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, Arg, Ala, Ser, and Lys;

X2 is selected from Gly, Glu, Ala, Val, Thr, and Asp;

X3 is selected from Glu, Thr, Gly, Asp, or Pro;

X4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;

X5 is selected from Thr, Ser, Val, and Ala;

X6 is selected from Arg, Val, Lys, Pro, Thr, and Asn; and

X7 is selected from Pro, Gly, Phe, Asn, and Arg; and

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

[0006b] In another aspect, there is provided a pharmaceutical composition comprising:

a) a recombinant adeno-associated virus virion described herein; and

b) a pharmaceutically acceptable excipient.

[0006c] In another aspect, there is provided use of a recombinant adeno-associated virus (rAAV) virion described herein in the manufacture of a medicament for delivering a gene product to a retinal cell in an individual.

[0006d] In another aspect, there is provided use of a recombinant adeno-associated virus (rAAV) virion described herein in the manufacture of a medicament for treating a retinal disease in an individual.

[0006e] In another aspect, there is provided 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 a peptide insertion relative to a corresponding parental AAV capsid protein,

wherein the insertion site is located between two adjacent amino acids at a position between amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype,

wherein the peptide insertion is a peptide of Formula I, Formula IIa, Formula IIb, Formula III, or Formula IV, wherein:

i) Formula I is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, and Lys;

X2 is selected from Gly, Glu, Ala, and Asp;

X3 is selected from Glu, Thr, Gly, and Pro;

X4 is selected from Thr, Ile, Gln, and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Arg, Asn, and Thr; and

X7, if present, is selected from Pro and Asn;

ii) Formula IIa is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

each of X1 – X4 is any amino acid;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iii) Formula IIb is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu and Asn;

X2, if present, is selected from Gly and Glu;

X3 is selected from Glu and Thr;

X4 is selected from Thr and Ile;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iv) Formula III is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is Lys;

X2 is selected from Ala and Asp;

X3 is selected from Gly and Pro;

X4 is selected from Gln and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Asn and Thr; and

X7, if present, is Asn; and

v) Formula IV is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, Arg, Ala, Ser, and Lys;

X2 is selected from Gly, Glu, Ala, Val, Thr, and Asp;

X3 is selected from Glu, Thr, Gly, Asp, or Pro;

X4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;

X5 is selected from Thr, Ser, Val, and Ala;

X6 is selected from Arg, Val, Lys, Pro, Thr, and Asn; and

X7 is selected from Pro, Gly, Phe, Asn, and Arg,

and wherein the AAV variant capsid protein, when present in an AAV virion, provides for increased infectivity of the AAV virion of a retinal cell.

[0006f] In another aspect, there is provided an isolated, genetically modified host cell comprising the nucleic acid described herein.

[0006g] In another aspect, there is provided a variant adeno-associated virus (AAV) capsid protein, wherein the variant AAV capsid protein comprises a peptide insertion relative to a corresponding parental AAV capsid protein, wherein the insertion site is located between two adjacent amino acids at a position between amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype, wherein the insertion is a peptide of Formula I, Formula IIa, Formula IIb, Formula III, or Formula IV, and wherein:

i) Formula I is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, and Lys;

X2 is selected from Gly, Glu, Ala, and Asp;

X3 is selected from Glu, Thr, Gly, and Pro;

X4 is selected from Thr, Ile, Gln, and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Arg, Asn, and Thr; and

X7, if present, is selected from Pro and Asn;

ii) Formula IIa is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

each of X1 – X4 is any amino acid;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iii) Formula IIb is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu and Asn;

X2, if present, is selected from Gly and Glu;

X3 is selected from Glu and Thr;

X4 is selected from Thr and Ile;

X5 is Thr;

X6 is Arg; and

X7 is Pro;

iv) Formula III is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is Lys;

X2 is selected from Ala and Asp;

X3 is selected from Gly and Pro;

X4 is selected from Gln and Lys;

X5 is selected from Thr and Ala;

X6 is selected from Asn and Thr; and

X7, if present, is Asn; and

v) Formula IV is $Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$, wherein:

each of Y1 –Y4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X1, if present, is selected from Leu, Asn, Arg, Ala, Ser, and Lys;

X2 is selected from Gly, Glu, Ala, Val, Thr, and Asp;

X3 is selected from Glu, Thr, Gly, Asp, or Pro;

X4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;

X5 is selected from Thr, Ser, Val, and Ala;

X6 is selected from Arg, Val, Lys, Pro, Thr, and Asn; and

X7 is selected from Pro, Gly, Phe, Asn, and Arg.

[0006h] In another aspect, there is provided a nucleic acid comprising a nucleotide sequence encoding the variant AAV capsid protein 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.