AAV vectors which evade antibody recognition (AAVe) are required. Such vectors will help a) expand the eligible cohort of patients suitable for AAV-based gene therapy and b) allow multiple, repeat administrations of AAV-based gene therapy vectors.

[0006] The present disclosure provides methods and compositions comprising an adeno-associated virus (AAV) capsid protein, comprising one or more amino acid substitutions, wherein the substitutions introduce into an AAV vector comprising these modified capsid proteins the ability to evade host antibodies.

SUMMARY

[0007] The present disclosure provides an adeno-associated virus (AAV) capsid proteins, comprising one or more amino acid modifications, wherein the one or more amino acid modifications modify one or more antigenic sites on the AAV capsid protein. In some examples wherein the AAV capsid proteins are incorporated into AAV vectors, the modification of the one or more antigenic sites results in evasion of neutralizing antibodies.

[0008] In some examples, the disclosure provides a recombinant adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises a substitution in an antigenic site of the AAV capsid protein, wherein the substitution has a sequence of any one of SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, 16, 17, 297, 298, 299, or 411-421. In some examples, the AAV capsid protein is of an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, AAVrh74, Avian AAV or Bovine AAV. In some examples, the AAV capsid protein is chimeric.

[0009] In some examples, the disclosure provides a recombinant AAV capsid protein, wherein the AAV capsid protein comprises an amino acid sequence that has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity, with any one of SEQ ID NOs: 18-80, 300-612, or 783-785.

[0009a] In one aspect, the present disclosure refers to a recombinant adeno-associated virus (AAV) capsid protein, wherein the AAV capsid protein comprises a sequence of SEQ ID NO: 49 having a first substitution, a second substitution, and a third substitution, wherein the first substitution is a replacement of amino acids corresponding to residues 454-460 of SEQ ID NO: 49 with SEQ ID NO: 9, the second substitution is a replacement of amino acids corresponding to residues 585-590 of SEQ ID NO: 49 with SEQ ID NO: 17, and the third substitution is a replacement of amino acids corresponding residues 493-500 of SEQ ID NO: 49 with any one of SEQ ID NOs: 10, 12, 14 and 15.

[0010] The present disclosure also provides a nucleotide sequence, or an expression vector comprising the same, that encodes one or more of the AAV capsid proteins of the disclosure. The present disclosure also provides a cell that comprises one or more nucleotide sequences or expression vectors of the disclosure.

[0010a] In one aspect, the present disclosure refers to a polynucleotide encoding the recombinant AAV capsid protein disclosed herein.
[0010b] In one aspect, the present disclosure refers to an expression vector, comprising the polynucleotide disclosed herein.
[0010c] In one aspect, the present disclosure refers to a cell, comprising the polynucleotide disclosed herein.
[0011] The present disclosure also provides an AAV capsid comprising the AAV capsid protein of this disclosure. Further provided herein is a viral vector comprising the AAV capsid of this disclosure as well as a composition comprising the AAV capsid protein, AAV capsid and/or viral vector of this disclosure in a pharmaceutically acceptable carrier.
[0011a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) vector, comprising the recombinant AAV capsid protein disclosed herein, and a cargo nucleic acid encapsidated by the AAV capsid.
[0012] The present disclosure additionally provides a method of introducing a nucleic acid into a cell in the presence of antibodies against the AAV capsid, comprising contacting the cell with the viral vector of this disclosure. The cell can be in a subject and in some examples, the subject can be a human subject.
[0012a] In one aspect, the present disclosure refers to a method of introducing a cargo nucleic acid molecule into a cell ex vivo, comprising contacting the cell with the AAV vector disclosed herein.
[0013] In some examples, a method of treating a patient in need thereof is provided, the method comprising administering to the patient a therapeutically effective amount of an AAV viral vector of the disclosure.
[0013a] In one aspect, the present disclosure refers to a pharmaceutical composition, comprising the AAV vector disclosed herein.
[0013b] In one aspect, the present disclosure refers to use of the AAV viral vector disclosed herein in the manufacture of a medicament for treating a patient in need thereof, wherein the patient has a liver disease or liver disorder.
[0014] These and other examples are addressed in more detail in the detailed description set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **Fig. 1A** and **Fig. 1B**. Bubble plots showing analysis of library diversity, directed evolution and enrichment of novel antigenic footprints. Parental (Fig. 1A) and evolved (Fig. 1B) AAV libraries were subjected to high-throughput sequencing using the Illumina MiSeq platform. Following analysis with a custom Perl script, enriched amino acid sequences were plotted. Each bubble represents a distinct capsid amino acid sequence with the radius of the bubble proportional to the number of reads for that variant in the respective library. The y-axis represents the absolute number of reads, transformed to log base 2. Data are spread along the x-axis for ease of visualization. The percent reduction in unique clones (93.4%) demonstrates that numerous “un-fit” sequences were removed after a first round of evolution.
[0016] **Fig. 2A, Fig. 2B, Fig. 2C,** and **Fig. 2D**. Fig. 2A is a bubble plot showing the parental (input) library for the first round of evolution. Fig. 2B is a bubble plot showing the output library for the first round of evolution, which was used as the input library for the second round of evolution. Fig. 2C is a bubble blot showing the output library for the second round of evolution, which was used as the input library for the third round of evolution. Fig. 2D is a bubble plot showing the output library for the third round of