ENGINEERED ADENO-ASSOCIATED VIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application no. 10202300688R, filed 13 March 2023, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of biotechnology. In particular, the present invention relates to engineering and/or attenuating adeno-associated viruses.

BACKGROUND OF THE INVENTION

[0003] Recombinant viral vectors based on adeno-associated virus (AAV) have emerged as an ideal delivery platform for *in vivo* gene transfer and genome editing therapies. AAV vectors are derived from wild-type viruses that naturally infect humans. As a consequence, depending on the AAV vector serotype, up to two-thirds of humans are seropositive to AAV, thereby impacting the use of AAV for gene transfection and transfer. Since AAV capsid structures are very similar with high amino acid sequence homology, the various AAV serotypes can encounter cross-reactivity to antibodies (i.e., naturally occurring AAV serotypes have conserved sequences that are neutralized by the same antibodies).

[0004] Thus, there is an unmet need for adeno-associated viruses that are capable of evading pre-existing anti-AAV antibodies.

SUMMARY

[0005] In one example, the present disclosure refers to a composition comprising at least one modified adeno-associated viral (AAV) capsid protein, wherein the composition is resistant to neutralisation or is not neutralised by human neutralising antibodies, wherein the at least one modified adeno-associated viral (AAV) capsid protein is modified at any one or more of residues 24 to 47, 48 to 97, 72 to 121, 120 to 167 and 168 to 217 of an adeno-associated viral (AAV) VP1 capsid protein or an ortholog thereof.

[0005a] In one aspect, the present disclosure refers to a composition comprising at least one modified adeno-associated viral (AAV) capsid protein, wherein the composition is resistant to neutralisation or is not neutralised by human neutralising antibodies, wherein the at least one modified adeno-associated viral (AAV) capsid protein comprises at least one mutation, and wherein the at least one mutation is at residue 213 of an adeno-associated viral (AAV) VP1 capsid protein or an ortholog thereof.

[0006] In another aspect, the present disclosure refers to a nucleic acid sequence encoding the composition described herein, or encoding the one or more modified adeno-associated viral (AAV) capsid proteins described herein.

[0007] In yet another aspect, the present disclosure refers to a vector comprising the nucleic acid sequence disclosed herein.

[0008] In a further aspect, the present disclosure refers to a host cell comprising the vector described herein or the nucleic acid sequence described herein.

[0008a] In another aspect, the present disclosure refers to an adeno-associated virus particle comprising the AAV capsid proteins disclosed herein.

[0009] In one aspect, the present disclosure refers to the composition disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, the host cell disclosed herein, or the adeno-associated virus particle disclosed herein, for use in gene therapy.

[0010] In yet another aspect, the present disclosure refers to a method of identifying an adeno-associated viral (AAV) capsid protein or peptide which is resistant to neutralisation or is not neutralised by human neutralising antibodies, the method comprising (a) contacting the adeno-associated viral capsid protein or peptide with sera known to contain adeno-associated virus neutralising antibodies, thereby allowing the neutralising antibodies to bind to an epitope comprised within the adeno-associated viral capsid protein or peptide; (b) removing the neutralising antibody-bound epitope from the adeno-associated viral capsid protein or peptide; and (c) identifying the adeno-associated viral capsid protein or peptide that did not bind to the neutralising antibodies.

[0011] In a further example, the present disclosure refers to a method of preventing an immune response to an engineered adeno-associated virus, the method comprising administering to a subject an engineered adeno-associated virus comprising at least one modified adeno-associated viral (AAV) capsid protein described herein.

[0011a] In another aspect, the present disclosure refers to use of an engineered adeno-associated virus comprising at least one modified adeno-associated viral (AAV) capsid protein described herein in the manufacture of a medicament for preventing an immune response to the engineered adeno-associated virus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0013] **FIG. 1** shows a summary of phage immunoprecipitation sequencing method. FIG. 1A shows an AAV and CRISPR-Cas phage library that were designed with protein sequences from UniProt and NCBI Protein database. The library consists of roughly 10 000 peptides tiles of 49 amino acids with 25 amino acid overlaps. The DNA sequences encoding the peptides tiles were synthesized, cloned, and packaged into T7 phages that display the encoded peptide on the phage surfaces. Next, the phage library was mixed and incubated with patient serum that contains antibodies. The antibodies bound to their target peptide sequences as displayed by the phages, and the complexes were enriched from the sample using protein A/G- coupled beads (IP – immunoprecipitation). After the immunoprecipitation, the genomes of phages bound to antibodies were amplified by polymerase chain reaction (PCR) and sequenced, identifying the peptide sequences bound by the antibodies. The results are represented by a heat map where darker shade represents higher level of antibodies recognizing particular epitope. Assessment of the library representation as an input library (FIG. 1B) and as a cloned library (FIG. 1C), before and after cloning and packaging, are shown. FIG. 1D shows the results of assessment of the library quality by Sanger sequencing of 20 randomly chosen clones.

[0014] **FIG. 2** shows the heatmap results of epitope-mapping of AAV serotypes by human antibodies using PhIP-seq. AAV epitopes recognized by antibodies in pooled serum sample (left) or an individual patient's serum (right) are shown.

CLAIMS

1. A composition comprising at least one modified adeno-associated viral (AAV) capsid protein, wherein the composition is resistant to neutralisation or is not neutralised by human neutralising antibodies, wherein the at least one modified adeno-associated viral (AAV) capsid protein comprises at least one mutation, and wherein the at least one mutation is at residue 213 of an adeno-associated viral (AAV) VP1 capsid protein or an ortholog thereof.

2. The composition of claim 1, wherein the at least one modified adeno-associated viral (AAV) capsid protein further comprises a mutation at residue 70.

3. The composition of claim 1, wherein the at least one modified adeno-associated viral (AAV) capsid protein further comprises one or more mutations at residues 70, 24 to 47, 48 to 69, 71 to 212, and 214 to 217.

4. The composition of claim 1, wherein the at least one modified adeno-associated viral (AAV) capsid protein further comprises a mutation at any one or more residues selected from the group consisting of residues 24 to 93.

5. The composition of claim 4, wherein the at least one modified adeno-associated viral (AAV) capsid protein further comprises a mutation at any one or more residues selected from the group consisting of residues 24 to 73 and 48 to 93.

6. The composition of any one of the preceding claims, wherein the at least one modified adeno-associated viral (AAV) capsid protein comprises at least one, at least two, at least 3 mutations or more.

7. The composition of any one of the preceding claims, wherein the mutation results in the modified adeno-associated viral (AAV) capsid protein being epitope masked.

8. The composition of any of the preceding claims, wherein the mutation is selected from the group consisting of a “gca” mutation (alanine), a “cgg” mutation (arginine), a “tct” mutation (serine), and a “gau” or “gac” mutation (aspartic acid).

9. The composition of claim 8, wherein the at least one mutation is present at residue D213, and wherein when the at least one modified AAV capsid protein further comprises one or more mutations, the one or more mutations is at any one or more of the residues selected from the group consisting of A70, R168, K169, R170, L171, N172, F173, G174, Q175, T176, G177, D178, A179,

D180, S181, V182, P183, D184, P185, Q186, P187, I188, G189, E190, P191A, P192, P195, A193, A194, P195, S196, G197, V198, G199, S200, L201, T202, M203, A204, A205, G206, G207, G208, A209, P210, M211, A212, N214, N215, E216, G217, N215, and combinations thereof.

10. The composition of claim 8 or 9, wherein the at least one mutation is D213A and wherein when the at least one modified AAV capsid protein further comprises one or more mutations, the one or more mutations is selected from the group consisting of A70R, R168A, K169R, R170A, L171A, N172A, F173A, G174A, Q175A, T176A, G177A, D178A, A179R, D180A, S181A, V182A, P183A, D184A, P185A, Q186A, P187A, I188A, G189A, E190A, P191A, P192A, P195D, A193R, A194R, P195A, S196A, G197A, V198A, G199A, S200A, L201A, T202A, M203A, A204R, A205R, AAV-DJ_G206A, G207A, G208A, A209R, P210A, M211A, A212R, N214A, N215A, E216A, G217A, N215S, and combinations thereof.

11. The composition of any one of claims 8 to 10, wherein the at least one mutation is a "gca" mutation, wherein the "gca" mutation is present at residue D213, and wherein when the at least one modified AAV capsid protein further comprises one or more mutations, the one or more mutations is at any one or more of the residues selected from the group consisting of R168, R170, L171, N172, F173, G174, Q175, T176, G177, D178, D180, S181, V182, P183, D184, P185, Q186, P187, I188, G189, E190, P191, P192, P195, S196, G197, V198, G199, S200, L201, T202, M203, G206, G207, G208, P210, M211, N214, N215, E216, G217, and combinations thereof.

12. The composition of any of the preceding claims, wherein the at least one modified adeno-associated viral (AAV) capsid protein is AAV-DJ_D213A, AAV2_D213A, AAV6_D213A, or AAV9_D213A, and wherein when the composition further comprises one or more modified AAV capsid proteins, the one or more modified AAV capsid proteins is selected from the group consisting of AAV-DJ_R168A, AAV-DJ_K169R, AAV-DJ_R170A, AAV-DJ_L171A, AAV-DJ_N172A, AAV-DJ_F173A, AAV-DJ_G174A, AAV-DJ_Q175A, AAV-DJ_T176A, AAV-DJ_G177A, AAV-DJ_D178A, AAV-DJ_A179R, AAV-DJ_D180A, AAV-DJ_S181A, AAV-DJ_V182A, AAV-DJ_P183A, AAV-DJ_D184A, AAV-DJ_P185A, AAV-DJ_Q186A, AAV-DJ_P187A, AAV-DJ_I188A, AAV-DJ_G189A, AAV-DJ_E190A, AAV-DJ_P191A, AAV-DJ_P192A, AAV-DJ_P195D, AAV-DJ_A193R, AAV-DJ_A194R, AAV-DJ_P195A, AAV-DJ_S196A, AAV-DJ_G197A, AAV-DJ_V198A, AAV-DJ_G199A, AAV-DJ_S200A, AAV-DJ_L201A, AAV-DJ_T202A, AAV-DJ_M203A, AAV-DJ_A204R, AAV-DJ_A205R, AAV-DJ_G206A, AAV-DJ_G207A, AAV-DJ_G208A, AAV-DJ_A209R, AAV-DJ_P210A, AAV-DJ_M211A, AAV-DJ_A212R, AAV-DJ_D213A, AAV-DJ_N214A, AAV-DJ_N215A, AAV-DJ_E216A, AAV-DJ_G217A, AAV-DJ_N215S, AAV2_D213A, AAV6_D213A, AAV9_D213A, AAV-DJ_A70R, and AAV-DJ_A70R_D213A.

13. The composition of any one of the preceding claims, wherein the at least one modified adeno-associated viral (AAV) capsid protein comprises SEQ ID NO: 52, or is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57, 58, and 59, and
wherein when the composition further comprises one or more modified AAV capsid proteins, the one or more modified AAV capsid proteins comprises or is selected from the group consisting of SEQ ID Nos: 30 to 57 and 61 to 62, or is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID Nos 1 to 29 and 58 to 60.

14. A nucleic acid sequence encoding the composition of any one of claims 1 to 11, or encoding the one or more modified adeno-associated viral (AAV) capsid proteins according to any one of claims 1 to 11.

15. A vector comprising the nucleic acid sequence of claim 12.

16. A host cell comprising the vector of claim 13 or the nucleic acid sequence of claim 12.

17. An adeno-associated virus particle comprising the AAV capsid proteins according to any one of claims 1 to 11.

18. The composition according to any one of claims 1 to 11, the nucleic acid of claim 12, the vector of claim 13, the host cell of claim 14, or the adeno-associated virus particle of claim 15, for use in gene therapy.

19. A method of identifying an adeno-associated viral (AAV) capsid protein or peptide which is resistant to neutralisation or is not neutralised by human neutralising antibodies, the method comprising
    a. contacting the adeno-associated viral capsid protein or peptide with sera known to contain adeno-associated virus neutralising antibodies, thereby allowing the neutralising antibodies to bind to an epitope comprised within the adeno-associated viral capsid protein or peptide;
    b. removing the neutralising antibody-bound epitope from the adeno-associated viral capsid protein or peptide; and
    c. identifying the adeno-associated viral capsid protein or peptide that did not bind to the neutralising antibodies.

20. The method of claim 17, wherein the adeno-associated viral (AAV) capsid protein or peptide is selected from the group consisting of AAV-DJ, AAV1, AAV2, AAV3, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 AAV13, or an ortholog thereof.

21. Use of an engineered adeno-associated virus comprising at least one modified adeno-associated viral (AAV) capsid protein according to claims 1 to 11 in the manufacture of a medicament for preventing an immune response to the engineered adeno-associated virus.