

CLAIMS

1. A variant adeno-associated virus (AAV) capsid protein, comprising an inserted polypeptide relative to a parental AAV capsid protein, wherein the inserted polypeptide comprises a polypeptide selected from any one of 1)-6) or any combination thereof:
 - 1) LGDTTRP (SEQ ID NO: 12) or LALGDTTRPA (SEQ ID NO: 18);
 - 2) LAETTRP (SEQ ID NO: 11) or LALAETTRPA (SEQ ID NO: 17);
 - 3) LGETTRN (SEQ ID NO: 13) or LALGETTRNA (SEQ ID NO: 19);
 - 4) KADTTKN (SEQ ID NO: 14) or LAKADTTKNA (SEQ ID NO: 20);
 - 5) KDDTTRN (SEQ ID NO: 15) or LAKDDTTRNA (SEQ ID NO: 21); and
 - 6) LADTTKN (SEQ ID NO: 16) or LALADTTKNA (SEQ ID NO: 22).
2. The variant AAV capsid protein according to claim 1, wherein the polypeptide of any one of 1)-6) or any combination thereof is located in a GH loop of the AAV capsid protein; preferably, follows any one of the amino acid residues between positions 570 to 611 of a VP1 encoded amino acid sequence of a parental AAV2 capsid protein; and more preferably, is located between positions 587 and 588 of the VP1 encoded amino acid sequence of the parental AAV2 capsid protein, or between positions 588 and 589 of a VP1 encoded amino acid sequence of a parental AAV9 capsid protein, or at a corresponding position of other parental AAV serotype capsid proteins.
3. The variant AAV capsid protein according to claim 1 or 2, further comprising an amino acid residue point mutation selected from one or more of 1L, 15P, 34A, 57D, 66K, 81Q, 101R, 109T, 144K, 144M, 164K, 176P, 188I, 196Y, 226E, 236V, 240T, 250S, 312K, 363L, 368H, 449D, 456K, 463Y, 472N, 484C, 524T, 535S, 551S, 593E, 698V, 708I, 719M, 721L and 735Q, wherein the mutation is at a position based on an AAV2 capsid protein set forth in SEQ ID NO: 1, or a corresponding position of other AAV serotype capsid proteins; preferably, the point mutation is 708I.
4. The variant AAV capsid protein according to any one of claims 1-3, having an amino acid sequence that is set forth in any one of SEQ ID NOs: 3-9 and 23-27 or has at least 90% or 95% sequence identity thereto.
5. A recombinant adeno-associated virus (rAAV) virion, comprising:
 - (a) the variant AAV capsid protein according to any one of claims 1-4, and
 - (b) a heterologous polynucleotide.
6. The rAAV virion according to claim 5, wherein the heterologous polynucleotide comprises a polynucleotide that encodes a gene product selected from the group consisting of a polypeptide, an interfering RNA and an aptamer;

the polypeptide is preferably an antibody or an antigen-binding fragment thereof or a fusion protein.

7. The rAAV virion according to claim 6, wherein the heterologous polynucleotide further comprises a polynucleotide selected from one or more of (a) to (g) below:

- (a) a 5' inverted terminal repeat (5' ITR) and/or a 3' inverted terminal repeat (3' ITR),
- (b) a 5' untranslated region (5' UTR) and/or a 3' untranslated region (3' UTR),
- (c) a promoter,
- (d) an enhancer,

- (e) a post-transcriptional regulatory element,
- (f) a polyadenylation signal (polyA), and
- (g) a Kozak sequence;

preferably, the heterologous polynucleotide further comprises a polynucleotide selected from one or more of (a) to (g) below:

- (a) a 5' ITR and/or a 3' ITR derived from AAV2,
- (b) a 5' UTR and/or a 3' UTR derived from Xenopus globin,
- (c) a CMV promoter,
- (d) a CMV enhancer,
- (e) WPRE,
- (f) SV40 polyA, and
- (g) a Kozak sequence.

8. The rAAV virion according to claim 7, wherein the heterologous polynucleotide comprises, from the 5' end to the 3' end, the following polynucleotides operably linked and arranged in the order of (a) to (h) below:

- (a) an enhancer,
- (b) a promoter,
- (c) a 5' UTR,
- (d) a Kozak sequence,
- (e) a polynucleotide encoding a gene product,
- (f) a 3' UTR,
- (g) WPRE, and
- (h) a polyA.

9. The rAAV virion according to any one of claims 6-8, wherein the polypeptide is a neuroprotective polypeptide, an anti-angiogenic polypeptide, a polypeptide that enhances retinal cell function;
preferably, the anti-angiogenic polypeptide is a VEGF antagonist;
more preferably, the VEGF antagonist is aflibercept.

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10. The rAAV virion according to any one of claims 5-9, wherein the heterologous

polynucleotide comprises a polynucleotide encoding aflibercept set forth in SEQ ID NO: 38;

preferably, the heterologous polynucleotide comprises a polynucleotide that is set forth in any one of SEQ ID NOs: 39-41 or has at least 90% or 95% sequence identity thereto.

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11. An isolated polynucleotide, encoding the variant AAV capsid protein according to any one of claims 1-4.

12. A vector, comprising:

- 10 (a) the isolated polynucleotide according to claim 11, or
(b) the isolated polynucleotide according to claim 11 and the heterologous polynucleotide according to any one of claims 5-10.

13. A host cell, comprising the vector according to claim 12.

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14. A pharmaceutical composition, comprising:

- (a) the rAAV virion according to any one of claims 5-10; and
(b) one or more pharmaceutically acceptable carriers, diluents, excipients or buffers.

- 20 15. The rAAV virion according to any one of claims 5-10, the heterologous polynucleotide according to any one of claims 5-10, or the pharmaceutical composition according to claim 14 for use in specifically infecting a retinal cell, wherein the retinal cell is to be infected by intraocular injection, preferably intravitreal injection or subretinal injection.

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16. Use of the rAAV virion according to any one of claims 5-10, the heterologous polynucleotide according to any one of claims 5-10, or the pharmaceutical composition according to claim 14, in the manufacture of a medicament for treating an eye-related disease,

- 30 preferably the medicament is to be administered by intravitreal injection or subretinal injection.

17. The use according to claim 16, wherein the eye-related disease is a retinal cell disease; preferably, the retinal cell disease is selected from the group consisting of diseases of photoreceptor cells, retinal ganglion cells, Muller cells, bipolar cells, amacrine cells, horizontal cells, and retinal pigment epithelial cells.

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18. The use according to claim 16 or 17, wherein the eye-related disease is selected from the group consisting of: retinitis pigmentosa, macular degeneration, wet AMD, dry AMD, retinal neovascularization, choroidal neovascularization, diabetic retinopathy, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion,

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branch retinal vein occlusion, diabetic macular edema, diabetic retinal ischemia, ischemic retinopathy, diabetic retinal edema, retinoschisis, glaucoma, Leber's congenital amaurosis, and/or achromatopsia.

- 5 19. A method for delivering a heterologous polynucleotide to a target cell *in vitro* and/or *in vivo*, comprising contacting the target cell with the rAAV virion according to any one of claims 5-10, the heterologous polynucleotide according to any one of claims 5-10, or the pharmaceutical composition according to claim 14.
- 10 20. An rAAV virion production system, comprising:
 (a) the isolated polynucleotide according to claim 11;
 (b) a heterologous polynucleotide encoding a gene product; and
 (c) a helper element, having sufficient AAV rep function and helper function to package
15 the heterologous polynucleotide encoding a gene product of (b) into the variant AAV capsid of (a).
21. A method for producing or preparing the rAAV virion according to any one of claims 5-10, comprising: introducing the vector according to claim 12 and a vector comprising a helper element into a producer cell, and packaging and purifying the rAAV virion.