Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy

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SUPPLEMENTARY MATERIALS AND METHODS

Estimation of neutralizing antibodies against S/T mutant AAV5 vectors

Heat inactivated serum samples from WT-AAV5 or S/T→A mutant AAV5 injected animals at a dose of $5x10^{10}$ vgs were assayed for the neutralizing antibody (NAb) titres as described previously[20] by the Immunology core at University of Pennsylvania. The NAb titer is reported as the highest plasma dilution that inhibited AAV transduction of Huh7 cells by 50% or more compared with that for the naive serum control.

Histological studies

Liver tissues from mock-injected or those injected with either WT-AAV5 or the best performing S/T→A mutant AAV5 vectors were collected 4 weeks post-injection, fixed in 10% buffered formalin and processed for microscopy. Three micron thick liver sections were cut and stained with hematoxylin and eosin. The degree of lobular and portal inflammation was scored (inflammation score, IS) by a pathologist, who was blinded to the experimental conditions. Inflammation scores were based on degree of lobular and portal inflammation and calculated based on the criteria, 0- no inflammation, 1- minimal inflammation, 2- mild inflammation, 3- moderate inflammation**.** Median score for each group (n=3) was calculated.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Schematic representation of the serine (S), threonine (T) and lysine (K) residues mutated in AAV1 and their conservation status across other AAV serotypes. VP1 protein sequences from AAV serotypes 1 through 10 were aligned by ClustalW and the conservation status of the each of the target site for mutagenesis is shown in red.

Supplementary Figure S2. Schematic representation of the serine (S), threonine (T) and lysine (K) residues mutated in AAV5 and their conservation status across other AAV serotypes. VP1 protein sequences from AAV serotypes 1 through 10 were aligned by ClustalW and the conservation status of the each of the target site for mutagenesis is shown in red.

Figure S1

AAV1-mutations	K137R	T251A	S277A	S499A	S526A	S663A	S669A
AAV1	GAKT	LPTY	GYST	NNSN	ASHK	FSAT	FASF
AAV2	PVKT	LPTY	GYST	NNSE	ASHK	FSAA	FASF
AAV3	AGET	LPTY	GYST	NNSN	ASHK	FSPA	FASF
AAV4	GAKT	LPTY	GFST	TGSD	ATAG	FSST	VNSF
AAV5	GAKT	LPSY	GYST	RASV	MTNN	FSDV	VSSF
AAV6	GAKT	LPTY	GYST	NNSN	ASHK	FSAT	FASF
AAV7	GAKT	LPTY	GYST	NNSN	ATHK	FTPA	FASF
AAV8	GAKT	LPTY	GYST	NNSN	ATHK	FNQS	LNSF
AAV9	AAKT	LPTY	GYST	NNSE	ASHK	FNKD	LNSF
AAV10	GAKT	LPTY	GYST	NNSN	ATHK	FSQA	LASF

Figure S2

AAV-5 mutations	K32R	S268A	S485A	S652A	S658A	T107A	T328A
AAV1	PKAN	GYST	NNSN	FSAT	FASF	DTSF	LTST
AAV2	PKPA	GYST	NNSE	FSAA	FASF	DTSF	LTST
AAV3	PKAN	GYST	NNSN	FSPA	FASF	DTSF	LTST
AAV4	PKAN	GFST	TGSD	FSST	VNSF	DTSF	LTST
AAV5	PKPN	GYST	RASV	FSDV	VSSF	DTSF	LTST
AAV6	PKAN	GYST	NNSN	FSAT	FASF	DTSF	LTST
AAV7	PKAN	GYST	NNSN	FTPA	FASF	DTSF	LTST
AAV8	PKAN	GYST	NNSN	FNQS	LNSF	DTSF	LTST
AAV9	PKAN	GYST	NNSE	FNKD	LNSF	DTSF	LTST
AAV10	PKAN	GYST	NNSN	FSQA	LASF	DTSF	LTST

SUPPLEMENTARY TABLES

Supplementary Table S1. Vector biodistribution in various organs in C57BL/6 mice 4-weeks after intravenous administration of WT or S/T/K mutant AAV5 vectors. Values are shown as mean (± SD) vector copy numbers per mouse diploid genome.

Vector	Muscle	Lung	Heart	Kidney	Spleen
WT-AAV5	0.0015 (±0.0001)	0.0082 (±0.001)	0.0005 (±0.0001)	0.0003 (±0.00008)	0.000081 (±0.000009)
S268A	0.0005 (±0.0015)	0.02335 (±0.009)	0.0031 (±0.0009)	0.000086 (±0.00001)	0.00003 (±0.00001)
S485A	0.0092 (±0.0007)	0.2934 (±0.02)	0.00201 (±0.001)	0.000072 (±0.00002)	0.000173 (±0.00006)
S652A	0.0037 (±0.015)	0.1045 (±0.09)	0.00055 (±0.00001)	0.00052 (±0.0001)	0.000216 (±0.00008)
S658A	0.0096 (±0.012)	0.1559 (±0.04)	0.0000954 (±0.0000003)	0.00055 (±0.0003)	0.000741 (±0.00006)
T107A	0.0043 (±0.0003)	0.049 (±0.008)	0.000818 (±0.00007)	0.000015 (±0.000009)	0.00021 (±0.00003)
T328A	0.0145 (±0.007)	0.187 (±0.03)	0.0002 (±0.00005)	0.00058 (±0.0001)	0.00006 (±0.000009)
K32R	0.00039 (±0.001)	0.05415 (±0.009)	0.0002 (±0.00008)	0.0011 (±0.0004)	0.0001 (±0.00008)

Supplementary Table S2: Neutralization antibody formation against wild type or mutant AAV5 vectors: Pooled serum samples from WT-AAV5 or S/T-AAV5 mutant injected mice (n=4 per group) 4-weeks after vector administration was analyzed. Heat inactivated serum samples were assayed for the neutralizing antibody (NAb) titers as described in the 'Supplementary Materials and Methods'. The NAb titer is reported as the highest plasma dilution that inhibited AAV5 transduction of Huh7 cells by 50% or more compared with that for the naive serum control. Limit of detection of the assay was 1/5 dilution.

Vector	**Reciprocal NAb titer**
Mock	<5
WT-AAV5	5120
S268A	5120
S652A	5120
S658A	5120
T107A	5120
Anti-AAV5 rabbit serum control	40960

Supplementary Table S3. Histological scores of liver samples obtained from C57BL/6 mice (n=4) four weeks post-injection with WT-AAV5 or mutant AAV5 vectors.

Injected Vector	**Median inflammation score**
Mock	0.5 (0-1)
WT-AAV5	0 (0) (0)
S268A-AAV5	0(0-1)
S652A-AAV5	0.5(0-1)
S658A-AAV5	0(0)
T107A-AAV5	0.5(0-1)

Supplementary Table S4: Details of primers used for site-directed mutagenesis of specific Serine/threonine to Alanine and Lysine to Arginine residues in AAV1 capsid

RESIDUE	SEQUENCE (5' to 3')	NUCLEOTIDE CHANGE	CHANGE IN RESTRICTION ENZYME
AAV1-S277A	Wild Type Primer Sequence:- CAACCACTACTTCGGCTACAGCACCCCCTGGGGGTATTTTG Mutant Primer Sequence:- CAACCACTACTTCGGCTACGCCACCCCATGGGGGTATTTTG	AGC→ GCC	C→A NcoI appears
AAV1-S499A	Wild Type Primer Sequence:- CAAAAACAGACAACAACAACAGCAATTTTACCTGGACTGGTG Mutant Primer Sequence:- CAAAAACAGACAACAACAACGCCAATTTTACCTGGACTGGTG	AGC ->GCC	No silent mutation
AAV1-S526A	Wild Type Primer Sequence:- GCACTGCTATGGCCTCACACAAAGACGAC Mutant Primer Sequence:- GCACTGCCATGGCCGCACACAAAGACGAC	TCA->GCA	T→C NcoI appears
AAV1-S663A	Wild Type Primer Sequence:- CGAATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTC Mutant Primer Sequence:- CGAATCCTCCCGCGGAGTTTGCAGCTACAAAGTTTGCTTC	TCA->GCA	G→C SacII appears
AAV1-S669A	Wild Type Primer Sequence:- TTTCAGCTACAAAGTTTGCTTCATTCATCACCCAATACTCC Mutant Primer Sequence:- TTTCAGCTACAAAGTTTGCTGCATTCATCACCCAATACTCC	TCA ->GCA	BsmI appears
AAV1-T251A	Wild Type Primer Sequence:- CCTGGGCCTTGCCCACCTACAATAACCACC Mutant Primer Sequence:- CCTGGGCCTTGCCGGCCTACAATAACCACC	ACC->GCC	C→G NaeI appears
AAV1-K137R	Wild Type Primer Sequence:- CTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAAC Mutant Primer Sequence:- CTGGTTGAGGAAGGCGCGAGAACGGCTCCTGGAAAGAAAC	AAG->AGA	T→G, BplI appears

Supplementary Table S5: Details of primers used for site-directed mutagenesis of specific Serine/threonine to Alanine and Lysine to Arginine residues in AAV5 capsid.

RESIDUE	SEQUENCE (5' to 3')	NUCLEOTIDE CHANGE	CHANGE IN RESTRICTION ENZYME
AAV5-S268A	Wild Type Primer Sequence:- AACGCCTACTTTGGATACAGCACCCCCTGGGGGTAC Mutant Primer Sequence:- GCCTACTTTGGATACGCCACCCCCTGGGGG	AGC->GCC	No silent mutations
AAV5-S485A	Wild Type Primer Sequence:- CGGGGTCAACCGCGCCAGTGTCAGCGCCTTCGCC Mutant Primer Sequence:- GGTCAACCGCGCCGCTGTCAGCGCCTTC	AGT->GCT	TSPR I disappears
AAV5-S652A	Wild Type Primer Sequence:- GAAATATCACCAGCTTCTCGGACGTGCCCGTCAGC Mutant Primer Sequence:- ATATCACCAGCTTCGCGGACGTGCCCGTC	TCG ->GCG	HPYI 88I disappears
AAV5-S658A	Wild Type Primer Sequence:- TCGGACGTGCCCGTCAGCAGCTTCATCACCCAGTACAG Mutant Primer Sequence:- GACGTGCCCGTCAGCGCCTTCATCACCCAGTA	AGC ->GCC	ALU I disappears
AAV5-K32R	Wild Type Primer Sequence:- AAGCGGGCCCACCGAAACCAAAACCCAATCAGCAG Mutant Primer Sequence:- CGGGCCCACCGAAACCAAGACCCAATCAG	AAA->AGA	No silent mutation
AAV5-T107A	Wild Type Primer Sequence:- AAGCTCGCCGACGACACATCCTTCGGGGAA Mutant Primer Sequence:- CTCGCCGACGACGCATCCTTCGGGG	ACA->GCA	MWO I appears
AAV5-T328A	Wild Type Primer Sequence:- CGCCAACAACCTCACCTCCACCGTCCAAGT Mutant Primer Sequence:- CGCCAACAACCTCGCCTCCACCGTCCA	ACC->GCC	HPH I disappears