Docket #: S18-520

Enhancing AAV-mediated gene targeting with ribonucleotide reductase inhibitors

Researchers in the Mark Kay group have identified a method to increase the rates of AAV vector-mediated HR (AAV-HR) in mammalian cells, increasing the efficiency of gene targeting rate

Background

Many gene targeting techniques, including the one using promoterless and nuclease-free adeno-associated virus (AAV)-mediated gene targeting vectors, depend on successful insertion of a corrected gene or gene with therapeutic function into a specific targeted genomic site. This targeted insertion depends upon homologous recombination (HR) that uses endogenous DNA repair pathway in host cells. However, HR occurs at very low rates in mammalian cells, which is currently one of major rare limitation to expand the clinical application of gene targeting therapies, especially in the case of AAV-mediated HR (AAV-HR) without use of programmable nuclease, such as CRISPR/Cas9.

Technology

Researchers in the Mark Kay group have identified a method to increase the rates of AAV vector-mediated HR (AAV-HR) in mammalian cells, increasing the efficiency of gene targeting rate. Both in vitro and in mouse models, treatment of cells with a ribonucleotide reductase inhibitor results in more efficient integration of therapeutic genes into a variety of target cell types.

A variety of ribonucleotide reductase inhibitors, including hydroxyurea, gemcitabine, and fludarabine, can be used to drive augmented integration. In animal studies, only a short-term treatment of RNR inhibitor shows significant increase of nuclease-free AAV-HR efficiency without causing obvious toxicity. In addition, the rate of CRISPR/Cas9-mediated targeted integration is also enhanced in vivo by the use of a

RNR inhibitor.

Related Technology
For AAVs targeted to the central nervous system (CNS), also see further work from the Kay lab to optimize AAV vectors for their ability to cross the blood brain barrier, where AAV variants have transduction efficiencies above that of AAV9. (S18.439). Stanford Docket S18-439-"AAV vectors for CNS delivery"

Applications

- Improved efficiency of existing vectors for gene targeting and gene editing both in vitro and in vivo
- Could address diseases previously not amenable to gene therapy, including neurodegenerative diseases

Advantages

- Can apply to variety of existing gene targeting technologies with or without use of programmable nucleases and both in vitro and in vivo

Publications

- Tsuji S, Stephens CJ, Bortuolussi G, Zhang F, Baj B, Jang H, de Alencastro G, Muro AF, Pekrun K, Kay MA. Fludarabine increases nuclease-free AAV- and CRISPR/Cas9-mediated homologous recombination in mice. Nature Biotechnol 2022 April 7. doi: 10.1038/s41587-022-01240-2

Patents

- Published Application: WO2021034776
- Published Application: 20220275403

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