Docket #: S22-435

Detection and prevention of unintended CRISPR/AAV-mediated concatemeric knockins

Stanford researchers have discovered using a novel assay that a large proportion of CRISPR/AAV modified cells contain hidden concatemeric knockins that affect gene expression, and therefore developed a strategy to reduce their occurrence.

The combination of AAV with CRISPR/Cas9 has proven to be a highly efficient strategy for site-specific genome editing. Typically, Cas9 ribonucleoprotein (RNP) is electroporated into cells and induces a double-stranded break at a target site in the genome, while AAV delivers single-stranded DNA repair templates into the nucleus. The cell then employs endogenous DNA repair machinery to fix the Cas9-induced break using the AAV-delivered DNA as a template. This strategy has been used to make both small changes and large insertions in the genome, enabling both cures for genetic disorders as well as immune cell engineering for the treatment of cancers and autoimmune disorders.

However, Stanford researchers recently discovered that a large proportion of cells (~50% or more) edited in this way contain target-site concatemeric knockins of the AAV genome. Critically, these concatemeric knockins greatly affect the level of gene expression but cannot be readily detected by commonly used assays. Researchers therefore developed novel strategies to detect these knockins as well as an approach to greatly decrease their occurrence.

Stage of Development
Preclinical: Researchers demonstrated a protocol to detect concatemeric knockins and reduce their occurrence by ~10-fold.

Applications

- Improved AAV/Cas9 mediated precision gene insertions
- Treatment of genetic disorders, including sickle cell disease, cystic fibrosis, and epidermolysis bullosa
- *Ex vivo* cell engineering, including CAR-T
- Detection of concatemeric viral vector insertions and other complex genotypes
- Prevention of concatemeric viral vector insertions

Advantages

- No existing methods to detect and reduce concatemeric knockins
- Protocol requires minimal modification to existing gene editing pipelines

Publications

- Fabian P. Suchy, Daiki Karigane, et al. (2024). Genome engineering with Cas9 and AAV repair templates generates frequent concatemeric insertions of viral vectors . Nature Biotechnology.

Patents

- Published Application: WO2025137259

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