Researchers engineer in vivo delivery system for prime editing, partially restoring vision in mice

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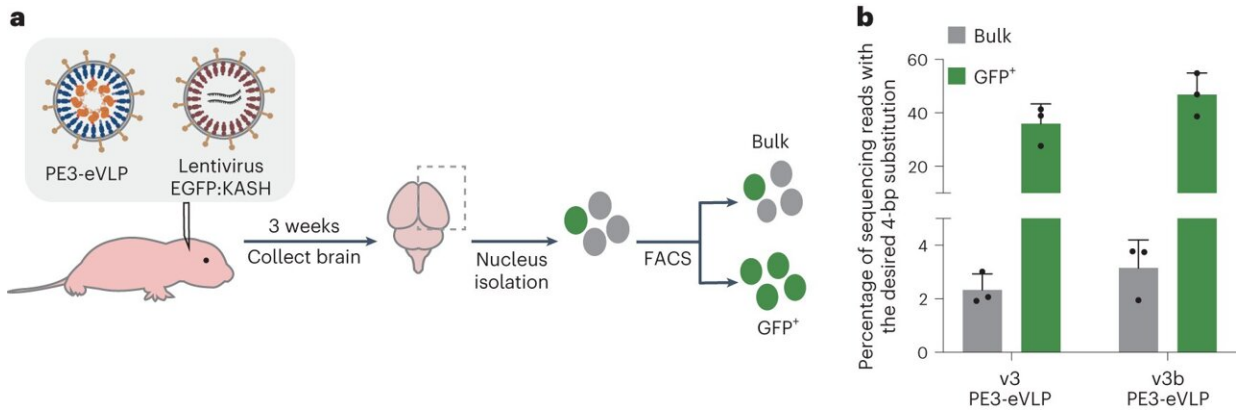

CNS editing with PE-eVLPs via P0 ICV injection. **a**, Schematic of workflow for neonatal ICV injection and subsequent analysis. FACS, fluorescence-activated cell sorting. **b**, Prime editing efficiency in bulk or GFP-positive population from the brain cortex collected 3 weeks following P0 ICV injection targeting the Dnmt1 locus with v3 PE3-eVLPs and v3b PE3-eVLPs. Bars represent the average prime editing efficiency of three mice and error bars represent the standard deviation, with each dot representing an individual mouse. Each mouse received approximately 1.0×10^{11} eVLPs in total. Credit: *Nature Biotechnology* (2024). DOI: 10.1038/s41587-023-02078-y

Prime editing, a versatile form of gene editing that can correct most known disease-causing genetic mutations, now has a new vehicle to

deliver its machinery into cells in living animals.

A team of researchers at the Broad Institute of MIT and Harvard has engineered virus-like particles to deliver prime editors to cells in mice at a high enough efficiency to rescue a genetic disorder. In the new work published in *Nature Biotechnology*, the team adapted engineered virus-like particles (eVLPs) that they had previously designed to carry base editors—another type of precision gene editor that makes single-letter changes in DNA.

Now the researchers describe how they re-engineered both eVLPs and parts of the prime editing protein and RNA machinery to boost editing efficiency up to 170 times in human cells compared to the previous eVLPs that deliver base editors.

The team used their new system to correct disease-causing mutations in the eyes of two mouse models of genetic blindness, partially restoring their vision. They also delivered prime editors to the mouse brain, and did not detect any off-target editing.

"This study represents the first time to our knowledge that delivery of protein-RNA complexes has been used to achieve therapeutic prime editing in an animal," said David Liu, senior author of the study and Richard Merkin Professor and director of the Merkin Institute of Transformative Technologies in Healthcare at the Broad. Liu is also a Howard Hughes Medical Institute investigator and a professor at Harvard University.

Delivery dilemma

Gene editing approaches promise to treat a range of diseases by precisely correcting genetic mutations that cause disease. Prime editing, described in 2019 by Liu's group, can make longer and more diverse types of DNA

changes than other types of editing. However, delivering the complex gene editing machinery to cells in living animals has been challenging.

The prime editing system has three components: a Cas9 protein that can nick DNA; an engineered prime editing guide RNA (pegRNA) that specifies the location of the edit and also contains the new edited sequence to install at that location; and a reverse transcriptase that uses the pegRNA as a template to make specific changes to the DNA.

Researchers have used a variety of methods to deliver these molecular machines to cells, including lipid nanoparticles and viruses. Virus-like particles (VLPs), composed of a shell of viral proteins that carry cargo but lack any viral genetic material, have also been of particular interest. But VLPs have traditionally yielded modest delivery outcomes in animals, and have to be specifically engineered for each different type of cargo to efficiently deliver to cells.

"We initially hoped that we could just take the eVLPs that we had painstakingly developed and optimized for base editing and apply them to prime editors," said Meirui An, a graduate student in the Liu lab and first author of the new paper. "But when we tried that, we observed almost no prime editing at all."

Bottleneck breakthroughs

In the new work, the researchers extensively re-engineered both the eVLP proteins and the prime editing machinery itself so that both the delivery and editing systems worked more efficiently. For instance, they improved how the prime editing cargo was packaged in the eVLPs, how it was separated from the delivery vehicle, and how it was delivered into the target cells' nuclei.

"The prime editor cargo must be efficiently packaged into eVLPs when

the particles form but must also be efficiently released from the particles after target cell entry," said Aditya Raguram, a former Liu lab graduate student and co-author of the study. "All of these steps have to be carefully orchestrated in order to achieve efficient eVLP-mediated prime editing."

While each individual improvement led to small jumps in the efficiency of the prime editors, the changes together had a much larger impact.

"When we combined everything together, we saw improvements of roughly 100-fold compared to the eVLPs that we started with," said Liu. "That kind of improvement in efficiency should be enough to give us therapeutically relevant levels of prime editing, but we didn't know for sure until we tested it in animals."

In vivo tests

Liu and his colleagues, in collaboration with Krzysztof Palczewski of the University of California, Irvine, first tested the system in mice to correct two different genetic mutations in the eyes. One mutation, in the gene Mfrp, causes a disease called retinitis pigmentosa that leads to progressive retinal degeneration. The other, in the gene Rpe65, is associated with blindness seen in the condition known as Leber congenital amaurosis (LCA) in humans.

In both instances, the eVLPs corrected the mutation in up to 20% of the animals' retina cells, partially restoring their vision.

The research group also showed that the eVLPs loaded with prime editing machinery could effectively edit genes in the brains of living mice. Nearly half of all cells in the cortex of the brain that received the editing machinery showed a gene edit.

"The gene editing field largely agrees that, moving into the future, gene editing machinery should ultimately be delivered as proteins to minimize potential side effects and we've now shown an effective way to do that," said Liu. "We plan to continue to actively work on improving eVLPs and adapting the technology to target other tissue types within the body."

More information: An, M. et al. Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo, *Nature Biotechnology* (2024). DOI: 10.1038/s41587-023-02078-y

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