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Enhancing base editor delivery

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Base editors (BEs) – which mediate targeted single-nucleotide conversions without requiring double-stranded DNA breaks – have the potential to correct the majority of known disease-causing single-nucleotide variants.

However, achieving safe and efficient in vivo delivery of BEs can be challenging. Writing in *Cell*, Banskota and Raguram et al. now describe the generation of

engineered DNA-free virus-like particles (VLPs), which efficiently and safely deliver therapeutic levels of BEs to mouse models of disease.

Existing approaches for delivering BEs in vivo commonly involve the use of viruses to deliver BE-encoding DNA to target tissues. However, this strategy is associated with various challenges, including the possibility of oncogenic viral vector integration into the genome of transduced cells. Since DNA-encoding editing agents are expressed for

a prolonged period in transduced cells, viral approaches also increase the risk of off-target editing. Lipid nanoparticles (LNPs) have also been used to deliver BE-encoding mRNA to the liver, but LNP delivery to other organs is more difficult.

Banskota and Raguram et al. therefore set out to investigate an alternative delivery approach: using VLPs to deliver BE ribonucleoproteins (RNPs). VLPs are assemblies of viral proteins that efficiently enter cells but lack viral genetic material, thereby preventing the possibility of causing a viral infection or viral vector genome integration. RNPs have a short lifetime in cells, which limits opportunities for off-target editing.

The authors hypothesized that retroviruses would be an attractive scaffold for engineering BE-VLPs: previous studies have indicated that certain retroviruses support VLP formation and even delivery of Cas9 RNPs in vitro, although achieving efficient in vivo editing using this approach has so far remained out of reach. Following generation of their initial BE-VLPs, Banskota and Raguram et al. identified key bottlenecks to VLP delivery efficiency. Extensive systematic engineering of the BE-VLPs to improve cargo release after VLP maturation, cargo localization and loading into VLPs, as well as component stoichiometry of VLPs, ultimately led to fourth-generation engineered VLPs (eVLPs), with 19-fold improved protein loading and up to 26-fold higher editing efficiency compared with initial designs.

In vitro and ex vivo studies demonstrated that eVLPs performed highly efficient on-target adenine base editing, with minimal off-target editing or DNA integration, in a variety of cell types, including multiple immortalized cell lines, primary human and mouse fibroblasts, and primary human T cells.

To begin to assess the in vivo efficacy of the eVLPs, the authors first investigated their

ability to perform base editing within the mouse CNS. eVLPs that install a silent mutation in mouse *Dnmt1* were delivered by neonatal cerebroventricular injection. Encouragingly, 3 weeks post-injection, 53% and 55% editing was observed in cortex and mid-brain cells that were exposed to the eVLPs.

Next, the authors investigated the ability of the eVLPs to mediate therapeutic base editing in adult animals. In mice, they first set out to disrupt the *Pcsk9* gene – a strategy for the treatment of familial hypercholesterolemia. A single intravenous injection of eVLPs targeting the boundary of *Pcsk9* exon 1 and intron 1, led to 63% editing in the liver, resulting in a 78% reduction in serum *Pcsk9* protein levels. Importantly, editing efficiency was comparable to that achieved with other state-of-the-art delivery systems, but without liver toxicity or other adverse effects that can be triggered by LNPs. Moreover, there was no detectable off-target editing, an improvement over existing viral delivery approaches.

Similarly, in an adult mouse model of the genetic retinal disorder Leber congenital amaurosis, a single subretinal injection of eVLPs efficiently and specifically corrected the disease-causing point mutation in *Rpe65*, leading to partial restoration of visual function.

Together, these findings establish eVLPs as a promising platform for efficient and safe in vivo delivery of therapeutic gene-editing agents.

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References

1. Banskota, S. et al. Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins. *Cell* <https://doi.org/10.1016/j.cell.2021.12.021> (2022)
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