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GENE THERAPY

In vivo gene editing in non-dividing cells

Targeted gene integration in non-dividing cells presents a great challenge, as existing genome editing techniques typically rely on the homology-directed repair (HDR) pathway, which is most active during DNA replication. In a new study published in *Nature*, scientists have developed a technique that enables DNA knock-in in non-dividing cells *in vivo* and used it to partially restore visual function in a rat model of retinitis pigmentosa — a hereditary degenerative disease that causes blindness.

The new approach — called homology independent targeted integration (HITI) — combines the CRISPR–Cas9 system with the cell's non-homologous end-joining (NHEJ) pathway for double-strand break (DSB) repair, which is active in dividing and non-dividing cells. Short sequences from the genomic target site are incorporated into a donor construct, where they flank the insertion cassette. The Cas9 enzyme is then targeted, via a guide RNA, to create DSBs at these sequences in the genomic target site and donor cassette, with the aim of subsequent NHEJ-mediated ligation of the insertion cassette into the genomic target site.

The researchers first evaluated and optimized HITI *in vitro* by using the system to introduce fluorescent protein genes at specific sites in dividing cells (a human embryonic kidney cell line) or non dividing cells (primary mouse neurons). In both cell types, targeted knock-in with HITI was highly efficient compared with other methods, including HDR.

Having established the *in vitro* efficiency of HITI, the team then tested it in live animals. To enable the targeting of non-dividing cells, they designed HITI constructs in which Cas9 expression could be induced using the drug tamoxifen. The constructs were delivered to mouse embryo brains by electroporation and Cas9 expression was induced 10 days after birth (when most nerve cells have ceased dividing). Immunofluorescence microscopy of mouse brain tissue 21 days after birth revealed efficient transgene insertion. Notably, these findings were recapitulated in the adult brain and other tissues in further experiments, demonstrating the versatility of HITI *in vivo*.

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The team then explored the potential of HITI for therapeutic gene replacement in an established rat model of retinitis pigmentosa. Adeno-associated virus (AAV) vectors, which have shown efficacy in humans, were used for HITI delivery. Three-week-old rats (in which retinal degradation has already started to occur) were injected in the subretinal space with a HITI–AAV construct designed to insert a missing exon from the *Mertk* gene (the causative mutation in this model). Analyses carried out 4 weeks after injection revealed increased levels of *Mertk* transcript and protein, and preservation of the thickness of the photoreceptor outer nuclear layer compared with control-treated animals. Moreover, electroretinography revealed a partial rescue of visual function following delivery of HITI; cone response was fourfold higher and overall photoreceptor activity was increased in treated compared with untreated eyes.

Finally, single-cell genotyping of mouse tissues demonstrated 90–95% on-target specificity following systemic delivery of HITI and biallelic integration of the transgene in 30–50% of cells. Encouragingly, sequence analysis of predicted off-target sites revealed minimal occurrence of insertions or deletions.

In enabling targeted transgene insertion into postmitotic cells *in vivo*, HITI should prove an invaluable tool in neuroscience research. Furthermore, it holds promise for a range of therapeutic gene-replacement applications, subject to further optimization.

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