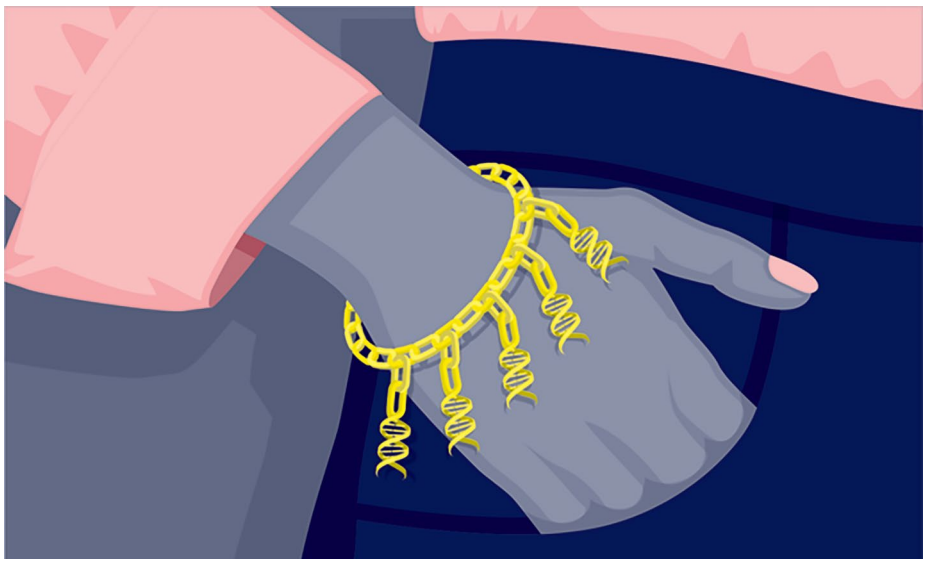
Gene therapy

Epigenetic editing works like a CHARM



Reducing the expression of pathogenic proteins holds great promise for the therapy of a wide range of human diseases. Epigenetic editing approaches are favoured over DNA-editing technologies, which have been associated with off-target sequence edits. However, current CRISPR-based epigenetic editors are too large for delivery in adeno-associated virus (AAV) vectors, which are approved for human use. Neumann, Bertozzi et al. describe a novel, more compact, epigenetic editor termed CHARM (coupled histone tail for autoinhibition release of methyltransferase) that mediates long-term transcriptional silencing in human cell lines in vitro and in mice, with minimal toxicity and off-target activity.

The authors used silencing of prion protein (PrP) expression in the brain — which can form toxic aggregates that cause neuronal death — to study the broader applicability of suppressing pathogenic proteins. Mice lacking *Prnp* (encoding PrP) are resistant to prion disease, and depletion of PrP expression in neurons after disease initiation is sufficient to prevent disease progression. Thus, reducing levels of PrP after symptom onset is a viable therapeutic strategy.

Neumann, Bertozzi et al. used a previously described CRISPRoff strategy to silence *PRNP* transcription in HEK293T cells by DNA methylation. CRISPRoff comprises a catalytically dead Cas9 protein fused to the catalytically active methyltransferase domain of DNMT3A (D3A) and the C-terminal domain of the activating co-factor DNM3TL (D3L), together with a single-guide RNA targeting the transcription start site of *PRNP*. *PRNP* remains durably silenced for up to 6 months after transient transfection, owing to extensive methylation across the promoter region. However, the CRISPRoff construct exceeds the packaging capacity of an AAV vector for in vivo use, and the bacterial enzyme Cas9, which can be chronically expressed from episomal AAV genomes, is likely to become antigenic. In addition, the D3A domain, which bypasses the autoinhibitory mechanism of full-length DNMT3A, is cytotoxic when overexpressed.

To overcome these issues, the authors investigated the use of zinc-finger proteins (ZFPs), which are smaller and less immunogenic, as an alternative DNA-targeting modality. They also developed a strategy to recruit endogenous DNMT3A, which is activated by binding to unmethylated histone H3, to remove the need to overexpress D3A. The CHARM construct allows for endogenous DNMT3A to be recruited to the methylation target site by a ZFP-fused D3L domain; methylase activity is induced by an unmethylated H3 tail fused to the N terminus of the construct.

Next, the authors sought to optimize various parameters of the CHARM construct. For connecting the H3 tail to the D3L domain, only a 40-amino-acid linker could achieve robust silencing of a target gene; modification of the sequence to increase flexibility moderately increased silencing activity. Testing a range of extant and ancestral D3L sequences showed that of the European wood mouse (*Apodemus sylvaticus*) to be most active. CHARM silencing activity was also increased by using a longer portion of the H3 tail, to increase affinity for DNMT3A.

Adding a synthetic, mutated ZFP-binding site to the promoter driving CHARM expression enabled heritable *Prnp* silencing followed by self-silencing of the CHARM construct through promoter methylation. This prevented chronic expression of the construct, and hence potential toxicity and immunogenicity, but without evidence of *Prnp* reactivation.

To reduce the size of the CHARM construct further and potentially allow for multiplex targeting, the authors used a split *Nostoc punctiforme* (Npu) intein strategy. Npu inteins separate the CHARM effector from the ZFP domain to generate two polypeptides from the same mRNA that can then be spliced together to form the complete, targeted effector. Thus, distinct DNA-binding domains targeting different genes could be encoded with a single CHARM effector within a single AAV.

Using previously published ZFPs targeting the mouse *Prnp* promoter, the team showed that the CHARM construct is better tolerated than CRISPRoff in terms of cell viability of transiently transfected cells, with near-complete silencing of *Prnp* transcripts and minimal off-target gene repression. In mice, intravenous administration of AAV-vectored, self-silencing CHARM constructs resulted in a marked decrease in *Prnp* transcripts and PrP protein in the brain after 6 weeks, with gene silencing in almost all neurons and no evidence of adverse effects. Silencing was associated with DNA methylation of the *Prnp* transcription start site.

The CHARM epigenetic editor is the first AAV-delivered tool for gene silencing by DNA methylation. It is suitable for multiplexed targeting and compatible with other DNA-binding modalities and other delivery platforms, thus being a broadly applicable strategy for therapeutic targeting of protein expression.

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