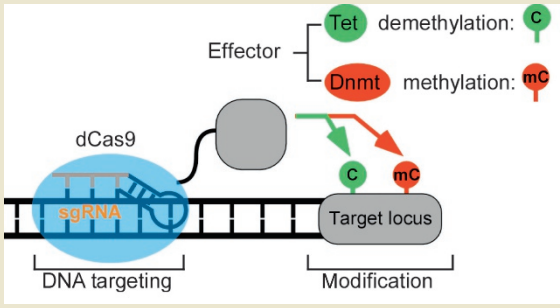
Epigenome editing to the rescue

Epigenetic dysregulation of gene expression is a major contributor to many human diseases. Targeted alteration of the epigenome became possible with the advent of customizable DNA binding domains, and the approach was quickly tested in animal models[1] and in clinical trials. In recent years, the development of easily programmable genome editors based on CRISPR–Cas9 has renewed interest in epigenome editing technologies and their therapeutic applications[2]. Now, a new study in *Cell*[3] has demonstrated the power of using specific editing of epigenomic marks to reverse the effects of a genetic mutation.



Reproduced with permission from Liu, X.S. *et al. Cell* **172**, 979–992.e6 (2018)

Epigenome editing makes use of the same customizable DNA binders (zinc finger proteins, TALEs or CRISPR–Cas9) that are used for genome editing or for general transcriptional activation or repression. But instead of being fused to a nuclease or to a transcriptional activator or repressor, the DNA binder carries an enzyme that puts in place or erases a specific epigenetic mark[2]. Although epigenome editors have been a boon to scientists investigating the mechanisms of epigenetic regulation, their utility for therapeutic purposes has not yet been tested.

As a first step toward therapeutic epigenome editing, Liu *et al.*[3] studied a Cas9-based DNA demethylase in a model of fragile X syndrome. This condition affects about 1:3,600 males and is the most common cause of male intellectual disability. It is caused by a trinucleotide repeat expansion in the 5′ UTR of the *FMR1* gene. In individuals with more than 200 of these repeats, the repeat region is hypermethylated, leading to formation of heterochromatin at the gene promoter and gene silencing.

To reverse the hypermethylation, the authors designed single-guide RNAs that target a catalytically inactive Cas9, which has been fused to the catalytic domain of the DNA methylcytosine dioxygenase TET1, to the hypermethylated repeats. Testing the constructs in patient-derived induced pluripotent stem cells (iPSCs), they observed a 96% reduction in the methylation levels of the repeats and an almost complete restoration of FMR1 expression. "A surprising and important finding was that the reversal of repeat methylation was so closely linked to the removal of heterochromatin marks and the appearance of active chromatin marks at the promoter," says Charles Gersbach, professor of biomedical engineering at Duke University in Durham, North Carolina.

Off-target demethylation events were rare. Although ChIP-seq detected >1,000 sites that were at least transiently bound by the demethylase, only 29 of these loci showed substantial demethylation. Of the 28 off-target genes affected, most showed no change, and none showed more than a fourfold change, in expression levels. "One of the lessons that we learned from gene therapy is that we have to carefully assess safety of the treatments before going into a clinical trial," says Angelo Lombardo of the San Raffaele Telethon Institute for Gene Therapy in Milan. "It is reassuring that the off-target effects observed here are limited, but one needs to keep in mind that epigenetic off-target effects might be much more context-dependent than genomic off-target events."

Restoration of FMR1 expression was sufficient to rescue cellular phenotypes associated with fragile X syndrome. Neurons that differentiated from methylation-edited iPSCs retained close to normal FMR1 expression levels and showed none of the electrical hyperactivity of affected neurons. Similar results were obtained if iPSC-derived neurons were treated after differentiation, although in this case demethylation and expression restoration remained incomplete. The authors also tested whether reactivation of *FMR1* is maintained *in vivo* by transplanting methylation-edited neural precursor cells into the brains of mice. After three months, about half of the neurons derived from the implanted cells still expressed FMR1. Interestingly, at least *in vitro*, maintaining expression of FMR1 did not require sustained dCas9–Tet1 activity. When the authors expressed a Cas9 inhibitor, FMR1 expression was unchanged for at least two weeks.

Further characterization of the therapeutic effects of dCas9–Tet1 was complicated by the limitations of the available mouse models. Inserting the extended repeats into mouse *Fmr1* does not result in DNA hypermethylation or gene silencing. The limitations of the fragile X mouse models also make it difficult to assess whether post-natal reactivation of FMR1 will be sufficient to achieve a cure.

"While this is an important first step, it remains to be seen how well this approach will translate into clinical applications. Delivery is an important issue as the dCas9–Tet1 fusion is too large for commonly used AAV [adeno-associated virus] vectors, although it should be possible to split the construct and deliver it in separate vectors," says Lombardo. And Gersbach highlights the potential issue of continuous transgene expression: "Ideally, one would like to avoid long-term expression of the epigenome editing tool, but additional research is necessary to evaluate if a hit-and-run approach would work here."

Markus Elsner
Senior editor

1. Rebar, E.J. *et al. Nat. Med.* **8**, 1427–1432 (2002).
2. Thakore, P.I., Black, J.B., Hilton, I.B. & Gersbach, C.A. *Nat. Methods* **13**, 127–137 (2016).
3. Liu, X.S. *et al. Cell* **172**, 979–992.e6 (2018).