Fine-tuning epigenome editors

For the raft of new ventures developing epigenome editors, a compelling niche may be diseases of haploinsufficiency or genome imprinting that require exquisite control of gene expression.

In recent months, three biotech startups — Chroma Medicine, Tune Therapeutics, and Navega Therapeutics — have raised $167 million in funding, joining Sangamo Therapeutics and Encoded Therapeutics in the quest to make epigenome editing a clinical reality. These startups are building platforms primarily around catalytically inactive CRISPR systems hooked to effector domains that modulate gene expression. Unlike marketed epigenetic cancer drugs that act in a genome-wide manner with dose-limiting toxicities, the specificity of epigenome editors promises to open up a range of new indications beyond oncology. In a landscape crowded by small-molecule inhibitors, monoclonal antibodies, gene therapies, small interfering RNAs, antisense oligonucleotides and conventional gene and base editing, the ability of epigenome editors to restore genes silenced in disease in a tunable and durable manner may prove a key therapeutic niche.

'Epigenetics' — describing phenotypic variation not originating from changes in genotype — was coined 89 years ago by Conrad Waddington. Since then, great strides have been made in understanding epigenetic mechanisms for gene control, such as chromatin remodeling, DNA methylation at CpG islands, and post-translational modifications (for example, methylation, acetylation, citrullination and phosphorylation) of the N-terminal tails protruding from the core histones that package genomic DNA.

Chromatin remodeling involves multiple factors, including ATP-dependent SWI/SNF, ISWI CHD/NURD/Mi-2 and INO80 family members, long intergenic non-coding RNAs, mRNAs and other proteins. Covalent chromatin modifications are carried out by enzymes termed 'writers' (for example, histone acetyltransferases, histone methyltransferases (EZH2 or DOT1L) or DNA methyltransferases), 'erasers' (for example, histone deacetylases, histone demethylases and TET proteins, which oxidize 5-methylcytosine in CpG dinucleotides), and 'readers' (for example, bromodomains, CW domains and DPF domains). By cooperating to control epigenetic marks, these enzymes can activate transcription (for example, via methylation of H3K4 (lysine 4 on histone H3) or acetylation of H3K9) or repress it (for example, via methylation of CpGs, H3K9 or H3K27). Projects like ENCODE and the Roadmap Epigenomics Mapping Consortium have accelerated our understanding of epigenetic states in health and disease, but the extent to which epigenetic alterations are drivers or consequences of disease often remains unclear.

This has not stopped drug makers from developing pharmaceuticals to reverse dysfunctional epigenetic states in oncology, the latest being Epizyme's Tazverik (tazemetostat), a small-molecule inhibitor of EZH2 for treating sarcoma and relapsed or refractory follicular lymphoma. All told, eight small-molecule epigenetic drugs are marketed. All act indiscriminately on epigenetic targets across the genome; and all show only mild efficacy, with dose-limiting toxicity associated with side effects like thrombocytopenia, neutropenia, nausea and even cardiac toxicity.

Hence, gene-specific epigenome editors offer alluring simplicity: a gene-specific DNA-binding domain — zinc-finger proteins (ZFPs), 'dead' Cas9 (dCas9) with mutations in its RuvC and HNH endonuclease domains, or transcription activator-like effectors (TALEs) — tethered via an amino acid linker to an enzymatic effector module. This effector is either an enzyme that directly places or removes a specific epigenetic modification (for example, TET, histone demethylases or the histone acetyltransferase p300) or a transcriptional activator (for example, VP16) or repressor (for example, KRAB).

A challenge is attaining durable epigenetic effects on gene expression over many cell divisions. With first-generation epigenome editors, induced transcriptional activity was often short lived following cessation of construct expression. Combinations of epigenome edits that are stably maintained over time and through cell divisions are thus needed. Long-term transcriptional repression has been achieved by H3K9 methylation and CpG methylation in cell culture, but long-term activation of gene expression has proven more difficult.

Similarly, although ex vivo delivery of epigenome editors via electroporation is possible, in vivo delivery beyond the liver and eye remains problematic (as for all macromolecular drugs). Although adeno-associated virus can accommodate ZFPs, dCas9 is too large to fit, although dCas9 variants with small effector domains can be delivered.

And while epigenome editing neither introduces permanent changes nor involves potentially genotoxic DNA breaks, it still poses safety challenges. Continuous expression or multiple dosing carries the risk of eliciting immune complications (here, mammalian ZFPs may have an advantage over bacterially derived dCas9 and TALE domains). Similarly, effector domains must be assessed for their ability to target proteins beyond chromatin: for example, p300 or CREB-binding protein can acetylate the oncoprotein p53.

Finally, given our rudimentary understanding of the complex interplay between different epigenetic processes, which modifiers in which combinations will deliver the desired clinical outcome? Epigenome editing has shown promise in a range of preclinical models, including diabetes, obesity, acute kidney disease, chronic pain and retinitis pigmentosa, as well as rare disorders like muscular dystrophy and Dravet syndrome. Two startups have disclosed their programs: Encoded is developing a DNA-binding domain tethered to an *SCN1A*-specific transcription factor under the control of a regulatory element specific to GABAergic inhibitory neurons to upregulate expression of the $Na_V1.1$ channel for Dravet syndrome, and Navega is investigating ZFPs or dCas9 targeted to *SCN9A* and fused to KRAB to repress the $Na_V1.7$ channel in chronic pain.

It will be a long and complex road, but epigenome editing therapies offer several unique therapeutic opportunities. They show promise for monogenic diseases where target genes exceed the payload capacity of AAV gene therapy and a healthy endogenous gene can be upregulated. But perhaps their most compelling therapeutic applications are restoring gene expression in congenital diseases of genome imprinting (for example, DiGeorge syndrome) and autosomal dominant diseases of haploinsufficiency. Here, the unique capacity to build epigenome editors with multiplexed effector domains promises a therapeutic modality that can fine-tune transcription and avoid toxicities associated with overexpression. ❐

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