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Review article

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Epigenetic editing: from concept to clinic

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Abstract

Dysregulated gene expression is associated with nearly all human disease and can be mediated by epigenetic modifications. Epigenetic editing aims to reprogramme gene expression by rewriting epigenetic signatures, without editing of the genome. Coined just over a decade ago, epigenetic editing initially faced major concerns regarding efficacy and specificity. Moreover, straightforward guidelines on how to induce sustained expression modulation for any given gene are still largely lacking. Yet, successes have been demonstrated in animal models of various diseases, and the first clinical trials of epigenetic editing have been initiated. Increased understanding of the mechanisms of epigenetic reprogramming is overcoming obstacles that initially hampered widespread application of epigenetic editing. Future advances in target specificity, reprogramming maintenance and delivery methods will enable epigenetic editing to become a powerful new therapeutic approach.

Sections

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Introduction

In merely a decade, the field of epigenetic editing (the gene-targeted overwriting of epigenetic modifications) has progressed from concept validation to clinical investigation. This development was catalysed by enormous multi-omics initiatives[1–4] that revealed that many diseases are associated with global changes in gene expression as well as epigenetic modifications on DNA (for example, cytosine methylation[5,6]) and the histone proteins associated with it[4] (Fig. 1a). These observations drove commercial development of small-molecule inhibitors of epigenetic writers, erasers, readers or remodellers[7] (Fig. 1b). Currently, there are ten FDA-approved epigenetic drugs (see Table 1), nine in the oncology space[8] and most recently one for Duchenne muscular dystrophy[9], with many more in development[10]. However, although effective mainly for some haematological malignancies, these drugs act broadly and transiently and are associated with toxicities[11] that limit widespread clinical application[10]. Controlled editing of epigenetic modifications at a given genomic locus offers an alternative approach that can stably reprogramme target gene expression, without intentionally affecting the whole epigenome and without altering the DNA sequence[12–14].

Although the concept of changing epigenetic modifications by recruiting a catalytic epigenetic effector to a given endogenous genomic locus in eukaryotic cells (Fig. 1c, left) was first reported in 2003 for histones[15] and DNA methylation[16], it remained relatively overlooked for another decade. Ten years later, when this approach was named epigenetic editing[17], only a handful of laboratories had reported on editing specific epigenetic modifications at endogenous loci to modulate target gene expression in mammalian cells[15,17–25], and the enormous promise of epigenetic editing to treat and possibly cure diseases was largely unrecognized. The development of CRISPR, an inexpensive, flexible and specific gene-targeting tool, fuelled a rapid increase in epigenetic editing studies. Indeed, the past decade has witnessed major academic and commercial investment in epigenetic editing, as both a research tool and a therapeutic strategy. Recent in vivo demonstrations of durable and efficient therapeutic gene silencing by epigenetic editing in mice and monkey models[26,27] (on a par with CRISPR knock-outs) are currently generating further interest in this technology.

Epigenetic editing companies are now emerging as pivotal players in the biotechnology and pharmaceutical industries (Table 2), dedicated to harnessing the power of epigenetic editing to develop innovative therapies for a wide range of diseases, such as cancer, neurodegenerative disorders and genetic diseases. Indeed, the first clinical trial of an epigenetic editing tool, OTX-2002 (Omega Therapeutics) was initiated in 2022[10]. OTX-2002 is an mRNA-encoded epigenetic modifier delivered by lipid nanoparticles (LNPs), designed to decrease expression of the *MYC* oncogene in hepatocellular carcinoma and other *MYC*-associated solid tumours. More recently, Tune Therapeutics initiated clinical trials in New Zealand and Hong Kong to test an epigenetic silencer to treat chronic hepatitis B and hepatitis D, and Epicrispr started a trial in the USA and New Zealand to treat facioscapulohumeral muscular dystrophy (FSHD) (Table 2).

Despite the enormous promise of epigenetic editing as a clinical strategy, caution is justified. The development of epigenetic editing as a therapeutic modality currently outpaces our knowledge of epigenetic regulation, which might lead to suboptimal agents being tested in initial clinical trials. Indeed, rules and tools for straightforward, effective, durable yet reversible, epigenetic editing are still largely unknown and likely context dependent. Moreover, safe clinical translation of epigenetic editing will face similar challenges to those encountered by DNA editing technologies, particularly regarding delivery[28].

In this Review, to offer a balanced discussion of the potentials and pitfalls of epigenetic editing, a historical overview of seminal studies demonstrating the potential of epigenetic editing by overcoming initially perceived limitations is first provided. Addressing these prior obstacles to epigenetic editing provides insightful knowledge to be carefully considered given the rapid clinical translation. Next, epigenetic editing in vivo studies, indicating the promise of targeting epigenetic writers and/or erasers to genes of interest as a clinical approach to treat a wide variety of diseases, are reviewed. Special attention is given to delivery, one of the main challenges in the clinical development of epigenetic editing. Substantial academic and commercial investment in DNA editing approaches is poised to resolve at least some technical delivery hurdles. If given the time and resources to learn from clinical outcomes, such editing studies will pave the way for epigenetic editing to become a new player in the clinical arena.

Epigenetic editing tools

Genetic information remains stable overall in the vast majority of the cells in the human body, yet cell types are characterized by distinct cellular programmes, morphologies and functions. This remarkable ability is achieved through epigenetic mechanisms, which include regulated chemical modifications of DNA molecules and histones. Such mechanisms collectively constitute the epigenome and regulate nucleosome positioning, chromatin accessibility and gene expression in a mitotically stable manner.

Cytosine DNA methylation (5mC) is a covalent chemical modification to the cytosine building block in the genome, which, depending on its location, affects gene expression and as such directs differentiation programmes[29]. Its dysregulation has the potential to prompt dysfunction and has thus been extensively explored as a biomarker to diagnose diseases[1,30]. On the other hand, post-translational modifications of histones can also affect gene expression. For example, histone acetylation can recruit bromodomain-containing proteins involved in transcription initiation and chromatin remodelling[31]. Although technically more challenging[32], differential histone modifications have been investigated to serve as biomarkers for disease[4]. To validate dysregulated genes as therapeutic targets, various initiatives including gene-targeting approaches were established to investigate the function of genes in their relevant context[33]. As epigenetic dysregulation is frequently associated with disease[1,34], epigenetic interventions to restore or modify gene expression profiles are promising for clinical applications.

Epigenetic editing can drive gene expression by writing active modifications that recruit the transcriptional machinery or increase chromatin accessibility (Fig. 1d), or by erasing repressive modifications. Epigenetic editing to write repressive modifications silences gene expression either by directly blocking recognition of their target DNA sequences by transcription factors or by promoting chromatin compaction to restrict recruitment (Fig. 1e). In addition, epigenetic editing may drive secondary changes in the epigenome that synergize towards robust gene activation or silencing. For example, targeted recruitment of an eraser of active histone acetylation recruits endogenous writers of repressive histone methylation[35]. A great number of epigenetic writers and erasers have been studied[12–14,36], greatly contributing to knowledge of epigenetic mechanisms of gene regulation.

Epigenetic editors

Epigenetic editing can be defined as the locus-targeted overwriting of epigenetic modifications to change the functioning of a genomic locus.

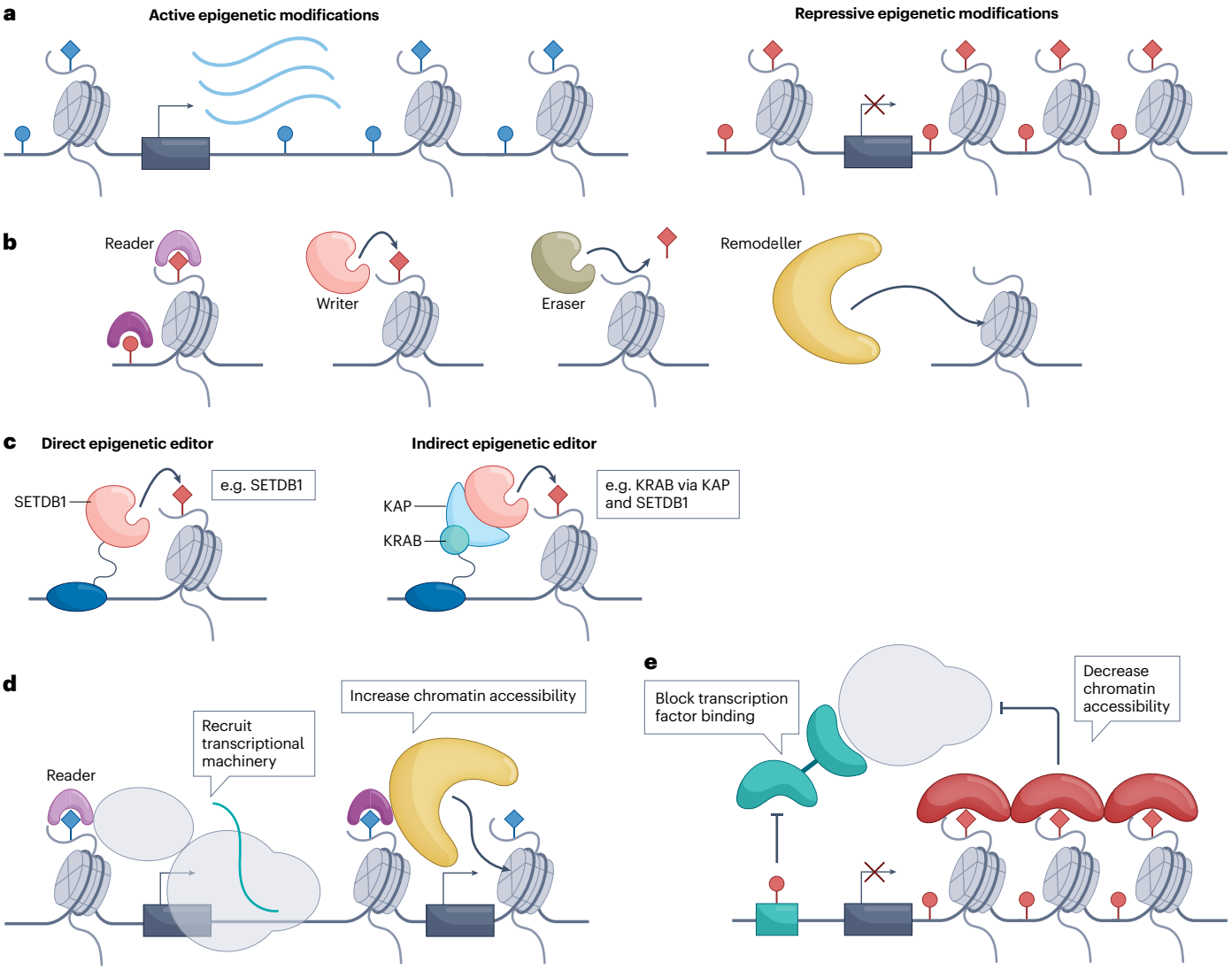

Fig. 1 | Epigenetic editing concepts. a, Epigenetic modifications are strongly associated with the activity of a gene: histone modifications (blue diamonds), such as histone acetylation (HAc) or histone 3 lysine 4 trimethylation (H3K4me3) at promoters (grey box with arrow), and DNA modifications (blue dots), such as cytosine hydroxymethylation or lack of cytosine methylation correlate with active genes (left panel); histone methylation of H3K9 or H3K27 (red diamonds) and DNA cytosine methylation (red dots) generally correlate with repressed genes (right panel). **b**, Epigenetic modifications are installed by enzymes called writers, removed by erasers and recognized by reader protein domains. In addition, chromatin remodellers can remove or exchange histones or reposition nucleosomes, thus affecting histone modifications or chromatin accessibility. **c**, Direct (left panel) versus indirect (right panel) epigenetic editors detailing that a DNA binding domain can be directly fused either to an epigenetic writer or eraser (left) or to a protein domain that indirectly recruits such a catalytic effector (right panel) to change epigenetic modifications at a target locus. **d**, Example mechanisms by which chromatin modifications can drive gene activation. Left panel: reader domains (for example, bromodomains that recognize histone acetylation) increase recruitment of transcriptional machinery (transparent shapes); right panel: reader domains recruit chromatin remodellers that increase chromatin accessibility. **e**, Example mechanisms by which chromatin modifications can drive gene repression: DNA methylation can block binding of activating transcription factors (left panel); histone modifications such as H3K27me3 and H3K9me3 recruit readers that nucleate heterochromatin and decrease chromatin accessibility (for example, Polycomb proteins and heterochromatin protein 1), thus decreasing binding of transcription factors and transcriptional machinery (right panel).

To achieve this, an optimal epigenetic editor would consist of a small, non-immunogenic, easily programmable gene-specific DNA binding domain (DBD) fused to an epigenetic effector domain or combination of epigenetic effector domains that directly write or erase epigenetic modifications only at the targeted site. As such, epigenetic editing results in the rewriting of an epigenetic signature to dominantly and permanently reprogramme chromatin functioning (for example, gene expression) while maintaining the possibility of being reversed. This Review focuses on direct enzymatic mechanisms of action (Box 1), as opposed to equally promising gene-targeting expression modulation approaches that do not directly rewrite epigenetic modifications. Artificial transcription factors (ATFs) make up an important class of

Table 1 | FDA-approved small molecules that inhibit epigenetic enzymes (so called epigenetic drugs, epi-drugs)

Epidrug	inhibitor of	FDA approved
Vidaza	DNMT	2004
Decitabine	DNMT	2006
Vorinostat	HDAC	2006
Romidepsin	HDAC	2009
Belinostat	HDAC	2014
Panobinostat	HDAC	2015[a]
Enasidenib	IDH2	2017
Ivosidenib	IDH1	2018
Tazemetostat	EZH2	2020
Givinostat	HDAC	2024

In addition, valemetostat tosilate (an inhibitor of EZH1, EZH2) has been approved in Japan (PMDA). DNMT, DNA methyltransferase; HDAC, histone deacetylase. [a]Cancelled by the FDA in 2022, but remains EMA approved.

these indirect epigenetic editors, and some insights derived from their development are also described here as this has contributed to the optimization of direct epigenetic editors. Together with efficient and cell-specific delivery methods, epigenetic editing could provide a versatile tool to correct a multitude of pathophysiologies.

Programmable DNA binding domains. Various types of programmable DBD were initially explored, including synthetic polyamides and triplex-forming oligonucleotides[37,38], but these proved cumbersome for widespread application[39]. Engineered zinc finger proteins (ZFPs) and plant pathogen-derived transcription activator-like effectors (TALEs) dominated the field of programmable DBDs before the introduction of CRISPR[40–42] (Box 2). Designer ZFPs, based on the most abundant transcription factors in eukaryotes[43], are small (~30 amino acids per finger binding 3–4 bp), especially when compared with CRISPR–Cas9 (~1,000–1,400 amino acids, targeting 20 nucleotides), and generally consist of an array of three to six modular engineered zinc fingers[44]. TALEs are also relatively small (~33–35 amino acids per TAL repeat), with one repeat binding to one base pair in the target DNA[45]. Both ZFPs and TALEs require protein engineering in each repeat to achieve gene targeting. By comparison with ZFPs and TALEs, the relative ease and low cost of the CRISPR–Cas9 platform revolutionized targeted genome editing[41] (Box 2). The nuclease-deactivated version (dCas9) was reported soon after the introduction of CRISPR–Cas9 (ref. 46), making epigenetic editing widely accessible to researchers across a myriad of scientific domains.

Transcriptional effector domains and other indirect epigenetic editors that modulate gene expression. Artificial transcription factors (Box 3) consist of a programmable DBD fused to full-length proteins (or domains) that modulate gene expression without directly editing epigenetic modifications (Fig. 1c, right). Most widely explored are transcriptional effectors derived from viral or human transcription factors. Many transcriptional activators have been validated, including the viral VP16 tetramer VP64 (ref. 47), viral RTA[48] and domains from human transcription factors p65 (ref. 49) or NCOA3, FOXO3 and ZNF473 (ref. 50). Transcriptional repressors include KRAB domains from, for example, ZNF10 (ref. 47), ZIM3 (ref. 51) or ZNF705 (ref. 50). Recruitment of these ATFs can indirectly lead to epigenetic modifications by secondary recruitment of the endogenous epigenetic machinery, but these effects are less predictable and most ATFs generally act transiently[12,52,53]. For example, in a mouse model, silencing of an antisense transcript involved in Angelman syndrome required repeated delivery of ZF–KRAB to achieve long-term repression[54]. Similarly, the effects on target gene activation after transient ATF expression diminished over time[55,56]. As such, durable gene regulation requires long-term ATF expression, generally accomplished by delivery using lentiviruses, which integrate into the host genome, or adeno-associated virus (AAV), which is episomally maintained and only diluted upon cell division[57]. In this respect, a single AAV delivery to express a ZF–KRAB was sufficient to durably rescue behavioural phenotypes in the mouse model of Angelman syndrome[58]. However, permanent ATF expression obscures insight into indirect mechanisms that stabilize target gene regulation, including changes in chromatin[59,60].

Other indirect epigenetic editors that can modulate gene expression include chromatin readers, such as HP1 (refs. 61,62) and MeCP2 (ref. 63), as well as chromatin remodellers, such as BAF[55,61,64] (Fig. 1b). Indirect epigenetic editing can also be achieved by fusing the DBD to a portion of the unmodified histone H3 tail (H3K4me0) (termed coupled histone tail for autoinhibition release of methyltransferase (CHARM)), which recruits endogenous DNA methyltransferase 3A (DNMT3A) to enhance epigenetic memory upon KRAB-mediated silencing in dividing cells[53]. Although in non-dividing brain cells in vivo, KRAB was not needed for maintenance of DNA methylation-induced silencing, this combination KRAB–DNMT3A3L is generally used for hit-and-run treatments as detailed later. Of note, however, indirect epigenetic editing using KRAB and MeCP2 showed that maintenance can be achieved without DNA methylation[65]. In fact, targeting of KRAB alone to integrated reporter genes can lead to stable gene silencing through the recruitment of endogenous effectors (KAP1 and SETDB1, which writes H3K9me3) that promote heterochromatin formation[66–69]. Increasing the duration of KRAB recruitment or the number of KRAB effectors recruited to the target locus increases the size of the H3K9me3 heterochromatin domain[69,70], eventually leading to more DNA methylation (owing to endogenous feedback between H3K9me3 and DNA methylation[71]) and increased epigenetic memory[67,69,70]. Similarly, combinations of activation domains and SS18 (part of BAF) have shown some promise in indirectly inducing long-term epigenetic changes[55].

Direct epigenetic editors. Many enzymes have been identified that can write or erase epigenetic modifications. For most, the exact (context-dependent) functions are largely unknown, and their targeting to genomic loci allows unravelling of their effects. Currently, various direct catalytic epigenetic effectors have been validated[12–14], and include DNMT3A and DNMT3B, tet methylcytosine dioxygenases (TETs), histone acetyltransferase p300, histone-lysine *N*-methyltransferase G9A, histone deacetylase 3 (HDAC3) and lysine-specific histone demethylase 1 (LSD1). As epigenetic editors have the potential to make long-term changes to gene expression profiles without altering the DNA sequence, these tools are a potentially safer alternative to more irreversible genome editing strategies.

So far, combinations of indirect and direct catalytic epigenetic editors have resulted in the most straightforward approach for effective and sustained gene expression modulation[52,72–75]. This is also evidenced by recent in vivo work combining DNA methylation (written by DNMT3A) with the indirect effects of KRAB on histone modifications that result in long-term silencing[26,27,76], although DNA methylation might be sufficient

by itself in certain circumstances[53,77,78] (see also Box 4). Similarly, sustained activation of naturally silenced genes might require combinations of effectors, despite the fact that stable re-expression of artificially silenced genes was achieved by targeting TET only[27,52,75]. Recently, high-throughput approaches that tested thousands of combinatorial constructs in yeast[79] and human cells[80,81] identified novel combinations for epigenetic memory of silencing and activation. What follows is a comprehensive overview of the development of direct epigenetic editors, from insightful chromatin biology research tools to a new class of flexible therapeutics with a broad clinical application spectrum.

Table 2 | Epigenetic editing companies

Company	Technology[a]	Targeted diseases (genes)	Tool name, lead targets
nChroma Bio	CRISPR (DNA demethylation/methylation)	Allogeneic CAR-T cells (*B2M*, *RFXAP*, *CD3D*, *CD3G*, *HLA-E*); familial hypercholesterolaemia (*PCSK9*); hepatitis B (cccDNA, intDNA) and hepatitis D	CRMA-1001, cccDNA (NCT07200193, not yet recruiting)
CLAIRIgene	'All-in-one' AAV–CRISPR, DNA methylation	Alzheimer disease (*APOEe4*)	CLRI-002, *APOEe4*
Encoded Therapeutics	AAV-engineered transcription factor (eTF)	Dravet syndrome (*SCN1A*) Angelman syndrome (*UBE3*)	ETX101, *SCN1A* ETX201, *UBE3*
Epicrispr Biotechnologies	crisprMINI, gene expression modulation system (GEMS); 'suppress and replace'	Facioscapulohumeral muscular dystrophy (*DUX4*)	EPI-321[b], *DUX4* (NCT06907875) EPI-331, *UTRN*
		Duchenne muscular dystrophy (*UTRN*)	
		Retinitis pigmentosa 4 (*RHO*); retinitis pigmentosa 11 (*PRPF31*)	EPI-141, *RHO*
Epitor Therapeutics	Cas-Nano	X-reactivation and nucleotide repeat disorders; fragile X mental retardation 1 (*FMR1*)	Not disclosed
Epigenic Therapeutics	CRISPR and TALE EpiRegs; lipid nanoparticle delivery	Hepatitis B (cccDNA, intDNA)	EPI-003, cccDNA (NCT06745973, not yet recruiting)
		Hypercholesterolaemia (*PCSK9*)	EPI-001, *PCSK9*
		Ocular, neurodegenerative, metabolic, rare disease	–
Modalis	CRISPR–guide nucleotide-directed modulation (GNDM) AAV	Angelman syndrome (*UBE3*)	–MDL-206 (paternal *UBE3*)
		Dilated cardiomyopathy (*TTN*)	–MDL-105 (*TTN*)
		Congenital muscular dystrophy (*LAMA2*)	MDL-101, *LAMA2*
		Duchenne muscular dystrophy (*UTRN*)	MDL-201, *UTRN*
		Facioscapulohumeral muscular dystrophy (*DUX4*)	–MDL-103 (*DUX4*)
		Myotonic dystrophy type 1 (*DMPK*)	MDL-202, *DMPK*
Moonwalk Biosciences	EpiRead and EpiWrite: CRISPR-multiplexing	Not disclosed	Not disclosed
Navega Therapeutics	ZFP, CRISPR	Chronic pain (*SCN9A*)	Not disclosed
Flagship Omega Therapeutics	Omega epigenomic controllers: RNA-targeted insulators	Hepatocellular carcinoma (*MYC*)	OTX-2002[b], *MYC* (first clinical trial, not continued: NCT05497453)
Sangamo	Zinc finger repressor	Alzheimer disease (*MAPT*)	ST-501, *MAPT*
		Amyotrophic lateral sclerosis (*C9orf72*)	–
		Chronic pain, idiopathic small fibre neuropathy (*SCN9A*)	ST-503, *SCN9A*
		Huntington disease (*HTT*)	–
		Parkinson disease (*SNCA*)	ST-502, *SNCA*
		Prion disease (*PRNP*)	ST-506, *PRNP*
Scribe	Epigenetic long-term X repressors (ELXRs)	Dyslipidaemias (*PCSK9*)	STX-1150, *PCSK9*
Seelos Therapeutics	dCas9–DNMT3A	Parkinson disease (*SNCA*)	SLS-004, *SCNA*
Tune Therapeutics	ZFP, TALE, CRISPR, DNA methylation, activator and repressors (not disclosed)	Familial hypercholesterolaemia (*PCSK9*)	–
		Hepatitis B (cccDNA, intDNA)	TUNE-401[b], cccDNA (NCT06671093)

AAV, adeno-associated virus; cccDNA, covalently closed circular DNA; DNMT3A, DNA methyltransferase 3A; TALE, transcription activator-like effector; ZFP, zinc finger protein. [a]Technology: DNA binding domain, effector, and delivery method; as disclosed July 2025. [b]OTX-2002, Tune-401 and EPI-321 are currently the only epigenetic editing tools tested in clinical trials. All other tools listed in the table are in preclinical development.

Box 1 | Direct epigenetic editing

This Review uses the term direct epigenetic editing referring to the use of catalytic effector domains (epigenetic writers or erasers) that directly write or rewrite epigenetic modifications (Fig. 1c, left panel) fused to a programmable DNA binding domain (DBD, see Box 2) that targets specific DNA sequences. This strict definition focuses the article on studies reporting on the effect and maintenance of direct epigenetic overwriting of genomic loci. The comprehensive summary of in vivo studies (Table 3) thus excludes editing approaches that do not target a specific locus, such as those that use an endogenous transcription factor DBD[266] or an epigenetic reader protein to recruit a catalytic domain at many locations in the genome[267]. Moreover, this definition excludes indirect epigenetic effectors (Fig. 1c, right panel), including artificial transcription factors (ATFs), which can indirectly change the epigenetic signature of a locus by recruiting epigenetic enzymes (see Box 3). Although this article focuses on direct epigenetic editors, a few seminal studies using indirect epigenetic effectors that contributed important insights relevant to direct epigenetic editing and exemplify important progress in the clinical field are highlighted.

Considerations for efficient and safe direct epigenetic editing

When the term epigenetic editing was first introduced[17], the epigenetics field was still evolving as an independent discipline and the translation of epigenetic editing to the clinic was, at that time, generally conceived as unrealistic. Initial concerns related to, first, the sufficiency and efficacy of writing or erasing epigenetic marks by direct epigenetic editors in regulating endogenous gene expression; second, the locus specificity necessary for clinical application; third, the accessibility of epigenetically silenced genes to epigenetic editors, as these are tightly packed in 'closed' heterochromatin; and fourth, the stability of induced epigenetic reprogramming (Fig. 2). These potential pitfalls limited early adoption of epigenetic editing to only a few pioneering labs. Below, key studies that have addressed these perceived limitations of direct epigenetic editing are reviewed, while using examples of indirect epigenetic editing to highlight important developments.

Sufficiency and efficacy

Decades of research have established that changes in the epigenome are associated with changes in gene expression[82–84]. To prove causality, the enforced writing or erasing of epigenetic marks by direct epigenetic editing provides a powerful approach. In most cases, targeted editing of chromatin modifications is indeed sufficient to change gene expression[12–14,85,86]. However, it is not yet possible to predict whether epigenetic editing of a single epigenetic modification at a target locus in a given cell type will be sufficient to regulate target gene expression (Fig. 2a). This confusion stems from the limited knowledge of the mechanistic link between transcription and chromatin, and the complexity of the human gene regulatory network. In human cells, there are ~1,600 transcription factors and ~900 chromatin regulators[43,87], with extensive crosstalk between transcription and chromatin regulation. Moreover, different cell types express different subsets of these transcription factors and chromatin regulators, affecting the possible outcome of epigenetic editing.

Cause versus consequence. An important consideration in understanding epigenetic mechanisms is whether particular epigenetic modifications are the cause or consequence of gene expression[84,88–91]. For example, degradation of RNA polymerase II both halts transcription and depletes active histone modifications[90], while cellular differentiation induced by overexpression of Yamanaka transcription factors causes epigenetic changes before gene expression changes[92]. The lack of clarity on the function of each epigenetic modification is underscored by studies of H3K27 acetylation (H3K27ac), a modification typically associated with active gene expression. Although epigenetic editing to write acetylation at multiple lysine residues (including H3K27) was found sufficient to activate gene expression[60,93,94], genome-wide integration of an H3K27ac-defective mutant disrupted Polycomb-induced gene repression (mediated by H3K27me3) but had no effect on basal transcription[95]. The role of another activating mark (H3K4me3) might be to release paused RNA polymerase II during transcription elongation instead of initiating gene expression[96], explaining why writing of K4me3 is not always sufficient to induce gene expression[97,98]. These and other studies demonstrate that the mechanisms that connect individual epigenetic modifications with gene expression are still being deciphered; direct epigenetic editing can help to untangle them.

The first evidence of the efficacy of epigenetic writers was derived from targeting synthetic reporter constructs[17]. Although instructive, such constructs may be easier to silence than endogenous loci owing to possible synergy with transgene silencing[99]. Epigenetic editing to silence endogenous genes was pioneered in 2003 by VEGFA promoter-targeted ZF–G9a and ZF–Suv39H1 to enrich the repressive histone modification H3K9me3 (ref. 15). Repression of mammalian endogenous genes by epigenetic editing was not reproduced until a decade later, in studies targeting ZF–G9a to *Her2/Neu*[18], TALE–LSD1 to enhancers[24] and light-activated TALEs to *Neurog2* and *Grm2* (ref. 23). Activation of endogenous genes by epigenetic editing was achieved through writing of active modifications, such as histone acetylation by p300 (ref. 94) or CBP[100], or histone methylation at H3K4 by PRDM9 (ref. 97).

Beyond histones, epigenetic editing of DNA modifications has also been shown to be sufficient to repress or activate endogenous gene expression. Epigenetic editing to write DNA methylation and repress endogenous gene expression was first shown with ZF–DNMT3A[19] and in combination with DNMT3L (ZF–DNMT3A3L)[20]. Targeted DNA demethylation and associated gene activation was accomplished by recruitment of TET1 (ref. 22) or TET1/2/3 (ref. 25). Taken together, these early studies showed that writing or erasing DNA methylation at endogenous genes can indeed be instructive for changing gene expression. These first DNA methylation editing studies were quickly and substantially followed up[86,101]. In summary, research on endogenous genes confirms that epigenetic editing of DNA and histone modifications can causally regulate gene expression[12–14].

Context dependency. Although sufficient in many circumstances, studies have found that the efficacy of epigenetic editing depends on the target locus, epigenetic effector and cell type[50,98,102]. For example, epigenetic editing to write histone H3K27 methylation repressed gene expression in a locus-specific manner[73,102,103], and writing histone acetylation[94] or H3K4me3 (refs. 97,98) did not induce gene activation for all genes, despite successful editing. A systematic study of context dependency found among other things that epigenetic editing of H3K36me3 was effective when targeted to CTCF-containing loci and hindered by nearby YY1 transcription factor motifs[98]. Given such

complexity, a systematic approach is necessary to define the 'rules of engagement' with respect to both the editor, the target locus and the chromatin context[12–14,85,104–107]. Parameters necessary to predict the outcome of epigenetic editing include: effector domain efficacy in writing or erasing the target modification; accessibility of the genomic locus, including extent of local heterochromatin; and recruitment of endogenous transcription factors and chromatin regulators to the target site, both by readers of the modification and by the DNA sequence itself.

Early studies of epigenetic editing 'rules of engagement' capitalized on the promiscuous binding of some ZFPs to interrogate gene repression upon epigenetic editing of DNA methylation genome-wide[108,109]. As expected, increased DNA methylation repressed gene expression at many loci. However, the efficiency of editing DNA methylation and gene repression varied by locus. Lowly expressed genes were more amenable to DNA methylation than highly active genes[108]. Further, increased methylation of active transcription factor binding sites repressed gene expression, whereas methylation of repressive transcription factor binding sites led to gene upregulation[109]. Additional features contribute to the efficacy of DNA methylation editing, such as the density of CpGs[110], nucleosome positioning and the use of alternative transcription start sites (TSSs)[109].

Finally, to ensure that the effects on gene expression are a direct cause of the on-target epigenetic editing, key controls are essential. The selection of controls is complex and can include: first, DBD alone; second, DBD fused to a catalytically inactive effector domain; third, effector domain alone; and fourth, non-targeting or safe-locus binding DBD fused to the effector domain. The first and second controls can determine whether the DBD(-fusion) alone regulates gene expression by sterically interfering with transcription factor binding sites, histone and DNA-modifying enzymes[111], or nucleosomes that regulate chromatin accessibility. The second control is also important to rule out any indirect effects of promiscuous recruitment of other endogenous effectors. The third and fourth controls can determine recruitment of the effector domain to non-targeted endogenous sites that indirectly regulate the target locus. Key examples of promiscuous effector domains include p300, which recruits transcription factors via its non-catalytic domains[112], and catalytically inactive DNMT3A, which recruits endogenous DNMT3A via dimerization of its partner, DNMT3L[20,113]. Studies of LSD1 also underscore the complexity of effector domains and the need for multiple controls: although targeted recruitment of this histone demethylase erased H3K4 methylation and decommissioned active enhancers[24], catalytic inactivation of LSD1 had limited impact on gene expression genome-wide[114]. However, deletion of full-length LSD1 derepressed enhancers genome-wide, suggesting that LSD1 can repress independently of its catalytic demethylase function, by recruiting other chromatin regulators[114]. As such, the careful selection and design of control effectors is essential to define the direct effect of epigenetic editing on gene expression.

Choosing the optimal targeting location. Although it is clear that epigenetic editing is sufficient to regulate gene expression, additional studies are needed to define optimal target locations for each effector domain or combination. To identify the optimal targeting window around promoters, pioneering studies using the ATF dCas9–KRAB (CRISPR interference (CRISPRi)) used pooled growth screens with guide RNA (gRNA) tiling libraries targeting thousands of positions around many promoters[115,116]. These studies revealed that dCas9–KRAB is most effective when targeted within ~100 bp downstream of the TSS. In contrast, a similar pooled single guide RNA (sgRNA) tiling screen using the KRAB–dCas9–DNMT3A3L fusion (CRISPRoff, containing the direct writer of DNA methylation) showed it can silence efficiently when recruited as far as ~500 bp upstream or downstream of the TSS, thus showing a broader effective targeting window around promoters than KRAB alone[75].

Although most studies discussed so far modulated gene expression by targeting gene promoters, effective epigenetic editing can also occur at other gene regulatory elements, including insulators and enhancers[24,117,118]. As proof of concept, various high-throughput

Box 2 | Programmable DNA binding domains (ZFPs, TALEs, CRISPR)

Early programmable DNA binding domains (zinc finger proteins (ZFPs) and transcription activator-like effectors (TALEs)) were initially exploited to induce double strand breaks by fusing these proteins to nucleases. Although currently overshadowed by the flexible CRISPR platform, ZFPs and TALEs are still actively used and are being further developed, in both basic and clinical research settings[41], owing to their smaller size, lower immunogenicity and way of DNA binding (leaving the double strand intact). ZF nuclease and TALE nuclease fusions (ZFNs/TALENs) were used in foundational studies of targeted, locus-specific genome editing and set the stage for targeting of other effector domains. The introduction of CRISPR greatly advanced genome editing, owing to its intrinsic nuclease activity and facile low-cost reprogramming. CRISPR–Cas9 nuclease uses RNA-guided DNA targeting, which relies on nucleotide homologous base pairing and circumvents the need to engineer a new ZFP or TALE protein for each target DNA. CRISPR genome editing has already found clinical success, for example, addressing leukaemia and blindness[28]. Importantly, the first CRISPR drug (Casgevy) has recently been approved in the UK and the USA for treatment of sickle-cell disease and β-thalassaemia[268].

Further development of genome editing therapies, however, must be carefully monitored to avoid early failures that can set back the field, as occurred for gene therapy in the early 2000s. For example, toxicity has resulted in the death of some enrolled patients[28], and longer-term effects on immune response and DNA damage remain to be assessed. The most unpredictable toxicity of genome editing is the loss of large chromosomal regions owing to double strand breaks, but other unintended changes to the host DNA sequence cannot be excluded[234,269,270]. To overcome these limitations, the CRISPR–Cas9 nuclease has been mutated to prevent the introduction of double strand breaks, and instead only nicks the DNA on one strand. Fusion of Cas9 nickases to base editing and prime editing effectors provides potent alternatives for genome editing without double strand breaks (reviewed elsewhere[271,272]). Despite this advance, genotoxicity cannot be ruled out as DNA nicking still engages the DNA repair pathways[273]. Beyond toxicity in the patient, there are risks associated with heritability of on- and off-target genome engineering. Therefore, the nuclease-deactivated CRISPR–dCas9, which is exploited in epigenetic editing, might provide a safer alternative for clinical application.

Box 3 | Artificial transcription factors (indirect epigenetic editing)

To exploit gene targeting, without introducing changes to the genetic sequence, transcriptional activators or repressors have been fused to programmable DNA binding domains. Such artificial transcription factors (ATFs) have the potential to 'wake sleeping genes'[274], while 'repressing screaming genes'[275]. This is distinct from editing genetic sequences, which permanently alters the genome to generally completely knock out expression, or cDNA gene therapy, to (over) express transgenes. Although RNA-targeting approaches such as small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) are still currently used and optimized[276], ATFs provide advantages over these approaches, including: the ability to either upregulate or downregulate using the same technology, transient regulation of individual transcript isoforms from the endogenous site[277], partial knockdown or more physiological upregulation (versus overexpression) to tune the intervention effects.

The first ATF clinical trial was initiated two decades ago by Sangamo Biosciences. The transcriptional activator, ZF–p65, was targeted to *VEGFA*[278] and administered to patients with diabetes to induce angiogenesis and reduce neuropathy[279]. By 2008, the construct was tested in phase II clinical trials (diabetic neuropathy, stem cell mobilization, amyotrophic lateral sclerosis). Although *VEGFA*–ZF–p65 was well tolerated in patients, it did not confer therapeutic benefit compared with placebo-treated patients with diabetes, who did very well compared with those who received routine medical treatment. Further clinical investigations were halted[280] and focus was placed on zinc finger nucleases (ZFNs).

Fuelled by the versatility of the CRISPR platform, widespread interest rapidly arose to repurpose CRISPR for gene expression modulation[46,281]. CRISPR efficacy has been further improved through use of combinatorial single guide RNAs (sgRNAs) and effector domains, as well as novel modes of effector recruitment. Fusion of dCas9 to multiple effector domains increases target gene activation, such as in dCas9–VPR (VP64, p65 and RTA)[48], the synergistic activation mediator (SAM; MCP-p65-HSF1)[49] or NFZ (NCOA3, FOXO3, ZNF473)[50]. Other platforms include dCas9–affinity tag fusions that recruit antibody–effector fusions (SunTag[282], SSSavi[283] and sgRNA–MS2 stem–loop fusions that recruit stem–loop binding protein–effector fusions[284]). High-throughput screens have recently identified more compact and potent candidate transcriptional effector domains (40–85 amino acids)[51,56,285–288] that work efficiently at many loci[50] and could improve delivery of ATFs in translational applications[182,289]. ATFs have been important in pioneering in vivo therapeutic relevance[279,290], as well as in demonstrating allele-specific[122,291], multiplexed[126,292,293] and bidirectional gene control[294].

The efficacy of activating ATFs in vivo was demonstrated in studies of various pathologies[151]. For example, dCas9–SAM-induced activation of *Oct4* ameliorated hallmarks of ageing[295] and ZF–VP64 activation of *Psd95* was sufficient to rescue memory in mouse models of ageing and Alzheimer disease[124]. Activating ATFs may also prove effective in the treatment of neurodevelopmental disease, such as Dravet syndrome, for which AAV-based clinical trials have been initiated using ZF–VP64 based on promising outcomes in mice and non-human primates[296]. Targeted gene repression using ZF–KRAB or dCas9–KRAB ATFs[46,50,51] shows translational promise in, for example, metabolic[297], neurodegenerative[122,298] and neurodevelopmental disorders[58] as well as in improving antitumour activity of chimeric antigen receptor (CAR)-T cells[293].

approaches using indirect targeting via dCas9–KRAB have been used to identify enhancers and link them with their target genes[116,119–121]. However, the effects on gene expression upon targeting these gene regulatory elements often depend on the particular architecture and control logic of the locus. In fact, a recent study using bidirectional ATFs (CRISPR activation and inhibition (CRISPRai)) showed strong effects on gene expression when targeting a 'gatekeeper' or dominant enhancer, but weak effects when the gene was under the control of multiple redundant enhancers[93]. Although future research will determine the optimal locations and effector domains, there is no doubt that epigenetic editing is instructive for gene expression in many contexts.

Locus specificity

Locus-specific epigenetic editing (Fig. 2b) depends on features of both the DBD and the epigenetic effector domain. Although in silico design of ZFP and TALE DBDs is challenging, these tools have achieved target specificity in many studies, including in vivo[26,58,77,122–128]. Target specificity of ZFPs and TALEs is underscored by their application of gene editors in clinical settings. ZFP design has been further optimized by an innovative deep learning model trained on 49 billion protein–DNA interactions[129]. Alternatively, CRISPR uses a straightforward sgRNA design to facilitate gene-specific recruitment. Despite the ease of CRISPR design[75] targeting specificity is not assured[130,131] as sgRNA–DNA mismatches allow some off-target binding. To increase specificity of targeting, advances in CRISPR engineering include exploiting CRISPR variants[132], chemical modifications to the sgRNA[133], mutagenesis of the CRISPR platform[134] and off-target prediction algorithms[135]. However, these approaches primarily target the Cas9 nuclease and not the dCas9 used for epigenetic editing[136]. Eventually, ZFPs or TALEs may prove more clinically relevant than CRISPR[26,53,77,78,137–139] based on a smaller size, the mode of DNA binding (no disruption of the DNA helix) and a more favourable immunogenic profile with the first clinical epigenetic editing trial indeed exploiting the ZFP platform.

Many studies have demonstrated specificity of epigenetic editing, including dCas-mediated DNA methylation editing in primary human haematopoietic stem and progenitor cells (HSPCs)[140] and for ZFs fused to DNMT3A3L and KRAB in liver[26]. However, the choice of epigenetic effector also affects specificity[42,141], with some effectors causing genome-wide off-target modifications[142–144]. Off-target epigenetic editing inhibits cell growth and promotes cell death, which can prohibit the generation of stable cell lines expressing epigenetic editors, as reported for TET1, p300 and DNMT3A[52]. Indeed, it is well established that expression of the isolated catalytic domain of DNMT3A can be toxic in cells[53]. Fusion of only the catalytic core[94,145] or rational engineering of the effector domain[146] may improve efficacy compared with the full-length endogenous effector, but is not always effective in improving specificity[147]. Distal genomic interactions can also result in off-target epigenetic editing, although this can sometimes be a regulated consequence with beneficial effects. For example, targeted DNA methylation of the age-associated gene, *PDE4C* or *FHL2*, resulted

in methylation of other age-associated CpGs[148], potentially reinforcing functionality of the initial editing. Interesting demonstrations of the potential towards specificity include the achievement of allele-specific epigenetic editing[149,150]. These achievements together with innovations in protein engineering led to target specificity suitable for clinical application, especially important given that many conventional therapies, such as chemotherapeutic or epigenetic drugs, are far from target specific.

Accessibility of epigenetically silenced genes

Early studies of epigenetic editing addressed binding efficiency as a limitation for activation of epigenetically silenced genes, as heterochromatin may have limited accessibility for epigenetic editors (Fig. 2c). A large number of studies that addressed this potential limitation found that both ATFs and generally larger-sized direct epigenetic editors successfully activated silenced genes, including fetal genes and tumour suppressor genes[12–14,86,151]. Although these studies established that heterochromatin does not prevent access to epigenetic editors, it does affect kinetics[152], binding[153–156] and genome editing outcomes[157]. Indeed, combinatorial approaches, such as co-targeting a transcriptional activator[158] or treatment with epigenetic drugs[159], increased Cas9 activity at silenced, heterochromatic loci and can likely also be applied to improve efficacy of epigenetic editing. In fact, the large size of epigenetic effector fusions might hamper accessibility and efficacy of epigenetic editing. Approaches to address this include targeting of catalytic domains instead of full-length effectors[22], module-based effectors, such as SunTag and MS2 (ref. 74), and use of small DBDs like those derived from ZFPs[77] or CRISPR variants[130,160–162].

Stability of induced epigenetic reprogramming

A major promise of direct epigenetic editing is the potential for sustained gene regulation following transient expression of the epigenetic writer or eraser. Such sustained gene regulation can be as efficacious as CRISPR genome editing[26] but avoids the risks and side-effects associated with permanent genomic changes. Direct epigenetic editing has resulted in sustained gene silencing in specific instances (as detailed below), even in in vivo models (Box 4), yet it remains a challenge to predict which epigenetic edits will be maintained once the epigenetic editor is removed[73,163,164] (Fig. 2d). Unravelling exact mechanisms of epigenetic editing maintenance represents an active area of investigation in both basic and translational applications.

Assessing stability after epigenetic editing can be confounded when the target is a master regulator of cell identity, as is the case in models of cell differentiation[105]. In these scenarios, transient expression of a direct epigenetic editor (or even an ATF) can produce sustained effects when its target gene affects the global epigenome and gene regulatory network. For example, in embryonic stem cells, transient expression of the ATF dCas–VPR to activate only two genes was sufficient to induce self-organization into a reproducible embryo model[165]. Similarly, *Sox1* activation by the ATF dCas9–VP64 (ref. 166) induced neuronal differentiation. Notably, neuronal differentiation was more efficient when *Sox1* was targeted with both a DNA demethylase, dCas–TET1, and a transcriptional activator[166]. Although such outcomes offer great promise towards regenerative medicine[105], the sustained effects can be attributed to the initiation of cell differentiation programmes and do not provide insight into maintenance of epigenetic edits at the target locus.

Early studies indicated that promoter-targeted DNA methylation can result in sustained gene repression, although not all genes respond similarly. In models of breast cancer, sustained silencing of *SOX2* was achieved with ZF–DNMT3A[167] (but not by ZF–KRAB) and silencing of *CDKN2A*, *RASSF1*, *HIC1* and *PTEN* was maintained with dCas9–DNMT3A3L[168]. However, sustained effects were not observed after ZF–DNMT3A was targeted to *VEGFA*[169]. Genome-wide studies of DNA methylation using promiscuously-binding ZF–DNMT3A[108,109] provide further support that maintenance of DNA methylation is context dependent and delineate general features of this phenomenon. First, DNA methylation was maintained at ~25% of targets for at least 1 week. Second, maintenance was correlated with basal enrichment of repressive H3K27me3, which may synergize with targeted DNA methylation to achieve stable silencing[108]. Third, the rate of DNA methylation loss was variable, reflecting dynamic recruitment of transcription factor activators and TET demethylases[109]. An elegant way to demonstrate effective silencing in the presence of activating transcription factors, is to specifically write DNA methylation to silence a mutated allele, while allowing the wild-type allele to remain active. Although this

Box 4 | A pre-clinical milestone for epigenetic editing in hypercholesterolaemia

A series of groundbreaking studies targeting *PCSK9* have established epigenetic editing as a powerful therapeutic strategy for hypercholesterolaemia. PCSK9 promotes LDL receptor degradation, raising cholesterol levels. These studies harness targeted DNA methylation and indirect histone repression to silence *PCSK9*, offering durable gene regulation without altering DNA sequence.

The first demonstration of targeted in vivo silencing for cholesterol reduction used a CRISPR–dSaCas9–KRAB system delivered by adeno-associated virus (AAV) for long-term expression to repress *Pcsk9* in mice[297]. Serum cholesterol was reduced for at least 24 weeks. This was the first proof that transcriptional repression could function as a therapeutic approach for hypercholesterolaemia.

Combining KRAB with DNA methyltransferase 3A and 3L (DNMT3A3L) in zinc finger fusion (termed evolved engineered transcriptional repressor (EvoETR)) delivered as mRNA via lipid nanoparticles (LNPs) in a single dose reduced PCSK9 by ~50%, with effects lasting nearly 1 year — even after liver regeneration — demonstrating mitotic stability of the epigenetic marks[26].

Translating this technology into primates using LNP-delivered CRISPR–dCas9 fused to KRAB, DNMT3A and DNMT3L (DNMT3A3L), reduced circulating PCSK9 by ~90% and LDL cholesterol by ~70% in cynomolgus monkeys, with effects persisting for at least 1 year[27]. Strikingly, gene silencing was fully reversible using a dCas9–TET1 activator.

Interestingly, in vivo long-term silencing of PSCD9 has been confirmed in independent studies using another epigenetic effector (transcription activator-like effector (TALE)–MQ1) delivered as mRNA via LNP[78] and another Cas variant (OMEGAoff) delivered via AAV[76].

Together, these studies mark a turning point: programmable, durable and reversible gene silencing without genome modification is now progressing towards the clinic.

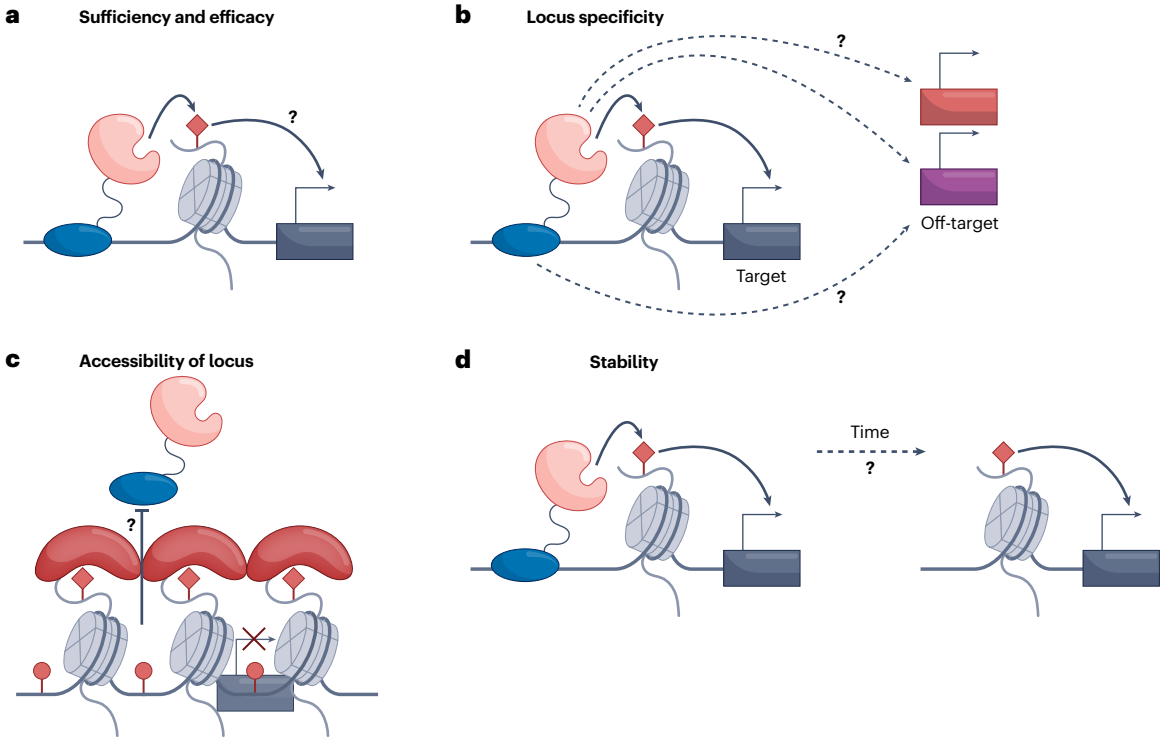

Fig. 2 | Considerations for efficient and safe epigenetic editing. a, Sufficiency and efficacy: a direct epigenetic editor (pink) fused to programmable DNA binding domain (blue) writes or erases epigenetic modification (red diamond) at a locus containing a gene promoter (grey box with arrow). The question remains: 'when is epigenetic editing sufficient and efficient in driving changes in gene expression (second curved arrow)?' **b**, Locus specificity: expression of an epigenetic editor could lead to writing of modifications and changes in gene expression at other genes (off-target), either by activity of its epigenetic editor (pink) or spurious binding of the DNA binding domain (purple). **c**, Accessibility: compacted heterochromatin containing repressive epigenetic modifications (red) could hamper access of the epigenetic editor to its target locus. **d**, Stability: after epigenetic editing at a locus, are the changes in epigenetic modifications and gene expression maintained over time in the absence of the epigenetic editor?

SNP-targeted silencing[149] (as well as activation[149,150]) was maintained at a subset of loci for days after dilution of the epigenetic editor, measurements over a longer period are needed to determine epigenetic memory. Such allele-specific epigenetic editing has many potential clinical applications, including silencing of dominant oncogenes[170] and correction of abnormal imprinting patterns[149,150].

Consistent with synergy of repressive pathways in maintaining epigenetic memory, combinatorial targeting of repressive epigenetic editors increases maintenance of gene silencing. A seminal study showed a synergistic effect on maintenance after combinatorial targeting of KRAB and DNMT3A3L to *B2M*, *IFNAR1* and *VEGFA*, using dCas9 or TALE DBDs in K562 cells[52]. Similarly, a KRAB–TALE–DNMT3A3L fusion targeted to *CXCR4* (ref. 163) and *PDCD1/LAG3* (ref. 139) resulted in sustained gene silencing in T cells, yet only transient silencing was obtained when targeted to the *CCR5* promoter, again suggesting that maintenance is context specific[163]. A breakthrough in the field was the introduction of CRISPRoff, the all-in-one KRAB–dCas9–DNMT3A3L fusion; the first genome-wide growth screen using this combination induced sustained repression at many genes[75]. However, some genes could not be stably silenced by transient CRISPRoff targeting[164]. Interestingly, exchanging dCas9–KRAB (associated with H3K9me3) with dCas9–Ezh2 (writing H3K37me3) could induce sustained effects at some dCas9–KRAB/dCas–DNMT3A and DNMT3L unresponsive genes[103]. Despite frequent inclusion of KRAB, direct epigenetic editing using only DNA methyltransferases showed that maintenance can be achieved in certain situations, also in vivo, including non-dividing brain cells[53] and liver[78].

Despite these success in maintenance of epigenetic editing-induced silencing[86] (see also Box 4), stable gene activation may prove more challenging as this requires not only the writing of activating marks and erasure of existing repressive marks, but also the binding of endogenous transcription factors to recruit transcriptional machinery and oppose heterochromatin formation. In fact, one effective strategy for sustained gene activation used editing of the promoter DNA sequence to introduce binding sites for endogenous transcription factors[171]. When endogenous activating transcription factors are already present, as is the case with genes synthetically silenced, for example, by CRISPRoff, epigenetic editing to remove DNA methylation using dCas9–TET1 can lead to sustained reactivation[52,75]. This approach was recently confirmed in mice, demonstrating reversibility of epigenetic gene silencing[27]. Although upregulation of genes silenced by DNA methylation is improved by co-recruitment of p65–RTA or VPR in addition to TET1, these indirect effectors do not greatly improve activation maintenance[75]. Stable reactivation induced by transient dCas9–TET1–CD expression was also confirmed for an enhancer involved in FOXP3 expression in human T cells, although this persistent demethylation status was not sufficient to induce a stable $CD4^+$ regulatory T cell phenotype[172]. In this respect, also in mouse primary T cells, lentiviral

TET1-induced demethylation of this enhancer region of *Foxp3* did not result in stable gene expression (in contrast to lentivirally expressed promoter-targeted dCas9–p300)[173]. Imprinted genes might be particularly permissive for maintenance of activation, as all transcription factors and cofactors necessary for expression are present and driving the sister allele. In this regard, a recent study proved that demethylating one allele of a repressed locus can result in prolonged gene re-expression[150]. A promising preclinical study of *SNRPN*, which is aberrantly imprinted in Prader–Willi syndrome, found that DNA demethylation via dCas9–TET1 maintained activation for 7 weeks, even across neuronal differentiation[174]. These examples show that removal of DNA methylation can result in sustained epigenetic gene activation in some cases.

In addition to DNA demethylation, epigenetic editing of active histone modifications can maintain transcriptional activation, but the effects are also context dependent. For example, transient recruitment of the H3K4me3 writer, PRDM9, to a gene without DNA methylation caused sustained gene activation, whereas targeting a DNA-methylated, silenced target gene caused only transient activation[97]. Interestingly, co-targeting of dCas9–PRDM9 with dCas9–DOT1L, which writes H3K79me2, to DNA-methylated loci seemed to improve maintenance[97]. In addition to locus variability, epigenetic memory of silencing and activation also varies with cell type[52,62,175], although the rules are yet to be determined. The availability of endogenous transcription factors and chromatin regulators necessary for maintenance are likely key. For example, a recent study[176] showed that deletion of chromatin factor DPPA2 in embryonic stem cells increases the durability of KRAB-mediated gene silencing.

Ultimately, published studies demonstrate that sustained epigenetic editing can be accomplished by specification of effector, locus and cell type. The challenge now is to predict this responsiveness, and efforts to find general rules and develop combinations of domains that work at specific (therapeutic) loci are still an active subject of research.

Therapeutic promise of direct epigenetic editing in animal studies

The studies discussed in the previous section highlight the power of epigenetic editing as a versatile in vitro research tool. However, efficacy in animal models has also been widely explored. In vivo epigenetic editing has been shown to be essential to definition of the direct causal relevance of a particular epigenetic modification to gene misregulation underlying disease; and to the distinction between the primary effects of epigenetic modification at a single locus and those due to genome-wide changes. Moreover, the promise of epigenetic editing as a therapeutic strategy has been demonstrated in many in vivo studies.

Below, the studies of epigenetic editing in vivo are reviewed, focusing on direct epigenetic editors. These studies may be divided into four classes (Table 3 and Fig. 3). Class I involves animal models derived from transgenic expression of the epigenetic editor. Class II involves animals derived from embryonic stem cells transiently treated with the epigenetic editor. Class III involves transplantation of cells that were reprogrammed ex vivo with epigenetic editors. Class IV involves epigenetic editing administered to disease models.

Class I and class II studies

Numerous transgenic mice have been engineered to constitutively or inducibly express dCas, but these approaches mainly exploit indirect epigenetic editors by targeting transcriptional activators, such as VP64 (ref. 177) or derivatives thereof (for example, mice with restricted expression of dCas–VPR to neurons[178] or cardiomyocytes[179]), or transcriptional repressors, such as KRAB[180], to regulate specific gene expression without directly modifying the epigenome (reviewed elsewhere[181,182]). One notable study combined a dCas9–KRAB transgenic mice model with viral expression of sgRNA–MS2 and MCP–LSD1, where MCP binds to MS2 sequences in the sgRNA molecule, to study enhancer function regulating haematopoiesis[121]. This tripartite inhibition system depleted the chromatin modification H3K4me1 and indirectly depleted H3K27ac while enriching for repressive H3K9me3, specifically at a targeted enhancer.

Alternatively, Cas9 transgenic mice have been exploited to study indirect epigenetic editing for gene expression modulation by delivery of truncated sgRNAs that prevent DNA cutting[183], for example, by recruiting HP1a–KRAB via the MS2 system to silence the myeloid differentiation primary response 88 (*Myd88*) gene[184]. This approach has also been applied for gene activation in a pig model with a Dox-inducible SpCas9 and truncated sgRNA using MS2–p65–HSF1 (MPH)[185]; pigs have competent immune systems closer to humans compared with rodents, so they represent valuable animal models. Transgenic models that express direct epigenetic editors would be valuable tools to study epigenetic regulation of disease-relevant genes in vivo, by overcoming the size limitations of viral delivery owing to the large size of epigenetic editors. To our knowledge, however, only a few transgenic animal models have been reported to assess the effects of direct epigenetic editor fusion constructs.

The first such transgenic models expressed dCas9–SunTag targeting *Tet1* to the *H19* gene to mimic Silver–Russell syndrome[186,187]. Similarly, a transgenic animal model was reported that exploits a neuron-specific promoter to restrict the expression of dCas–SunTag (recruiting DNMT3A) to neurons[188]. This model, upon expressing sgRNAs targeting *Snrpn*, was successfully used to alleviate the phenotype of Angelman syndrome. Another epigenetic editor transgenic model expressed Cre-dependent dCas9–p300, to allow temporal and regional control of dCas9–p300 expression[144]. Promisingly, AAV delivery of Cre and *Pdx1* sgRNA to dCas9–p300 mouse liver increased *Pdx1* mRNA and protein associated with H3K27Ac enrichment at the *Pdx1* locus[144]. However, off-target differential gene expression was reported and H3K27Ac enrichment was widespread, even in the absence of Cre or sgRNA expression[144]. This non-specific gene activation might be due to 'leaky' expression of the constitutively active catalytic domain of p300; other studies also reported on the promiscuous effects of p300 (ref. 98).

An alternative approach to generate epigenetic disease animal models is transient expression of epigenetic editors in early development to induce long-lasting epigenetic reprogramming of a gene of interest (class II in Table 3). Examples include targeted DNA demethylation or methylation to mimic imprinting disease[189,190], autism[191] and neurodevelopmental disorders[192], as reviewed elsewhere[86]. Excitingly, by simultaneously targeting eight locus imprinting control regions with dCas9–Dnmt3a and dCpf1–Tet1 to downregulate or upregulate their targets, respectively, viable offspring could be derived via mRNA co-delivery with their corresponding sgRNAs in unfertilized oocytes, albeit with low efficiency[193]. To our knowledge, no attempts were made to create animal disease models using direct histone epigenetic editing.

Four studies beyond conventional animal models shed light on using direct epigenetic editing to study species-specific epigenetic regulation mechanisms with the application of dCas9–Dnmt7 and dCas9–Tet2 in zebrafish embryo[194], dCas9–SunTag–DNMT3A in snail[195], dCas9–olEzh2 in Japanese killifish[196] or dCas9–LSD1 in chicken

Table 3 | in vivo direct epigenetic editing studies

	DBD	Effector domain	Target gene	Model/comments	Class[a]	Delivery	Ref.
Repression by DNA methylation	ZF	DNMT3A	*p16*	Cancer metastases	III	Transplantation of transduced cells	200
	ZF	DNMT3A	*SOX2*	Breast cancer	III	Transplantation of transduced cells	167
	ZF	DNMT3A3L (and KRAB)	*Pcsk9*	Hypercholesterolaemia — maintained (also through liver degeneration)	IV	LNP, i.v. delivery	26
	ZF	MQ1 (and KRAB)	*MYC*	Hepatocarcinoma (subcutaneous and orthotopic)	IV	LNP, i.v. delivery	137
	dCas9	MQ1(Q147L)	*Igf2/H19*	Imprinting	II	Plasmid-treated zygotes	189
	dCas9	DNMT3A	A^{vy}; *H19* ICR and *Dlk1–Dio3* IG-DMR; *Snrpn*	Coat colour; imprinting: bimother embryos; Angelman syndrome	II	mRNA microinjection in oocytes	190
	dCas9	DNMT3A3L	*Mecp2*	Autism	II	3×AAV/zygote microinjection	191
	dCas9	DNMT3A	*APP*	Alzheimer disease	IV	Lentivirus	226
	dCas9	DNMT3A	8 ICRs	Parthenogenesis (allele-specific, multiplexing)	II	mRNA microinjection in oocytes	193
	dCas9	DNMT3A	*Bace1*	Alzheimer disease	IV	Exosome injection	227
	dCas9	DNMT3A3L	*Ttr*	Mouse depression-like model	IV	jetPEI plasmid microinjection in prefrontal cortex	229
	dCas	DNMT3A3L	*P15*	Maintained through haematopoiesis	III	Reprogrammed human stem and progenitor cells	140
	dCas	DNMT3A	*SCNA*	Parkinson disease	IV	Injection of exosomes followed by focused ultrasound stimulation	228
	dCas–GCN10	DNMT3A	*Snrpn*	Angelman syndrome	I	Neuron-specific transgenic AS mice	188
	dCas9 (CRISPRoff)	DNMT3A3L (and KRAB)	*PSCK9* (humanized mice, NHPs)	Hypercholesterolaemia — maintained and reversible	IV	LNP i.v. delivery	27
	dCas9 (CRISPRoff)	DNMT3A3L (and KRAB)	*MGMT*	Glioblastoma	III	Intracerebral transplantation of treated cells (mRNA/sgRNA, electroporation)	205
	dIscB (OMEGAoff)	DNMT3A3L (and KRAB)	*Psck9*	Hypercholesterolaemia	IV	AAV8 i.v.	76
	TALE	MQ1	*Psck9*	Hypercholesterolaemia — maintained	IV	LNP	78
Upregulation by DNA demethylation	TALE	TET1	*Crmp4*	Prostate cancer metastases	III	Orthotopic transplantation of transduced cells	201
	TALE	TET1	*ICR2* (p57)	Diabetes	III	Transplantation of transduced β-cells	198
	dCas9	TET1	*Gfap*	Neurodevelopmental disorders	II	Electroporation and i.u. implantation	192
	dCas9	TET1	*Snrpn*–GFP	Dermis, brain — proof of concept	(IV)	Lentivirus	210
	dCas9	TET1	*Fmr1*	Fragile X syndrome	III	Transplantation of transduced cells	209
	dCas9	TET1	*SARI*	Colon cancer	III	Ectopic transplantation of transduced cells	202
	dCas9	TET1	A^{vy} (*H19/Dlk1; Snrpn Ube*)	Coat colour, imprinting: bimother embryos; Angelman syndrome	II	mRNA microinjection in oocytes	190
	dCas9	TET1	*ZNF154*	Oesophagal squamous carcinoma	III	Ectopic transplantation of transduced cells	203
	dCas9	TET1	*CDO1*	Breast cancer	III	Ectopic transplantation of transduced cells	204
	dCas9	TET1	*CTSD*	Alzheimer disease	IV	Lentivirus	225

Table 3 (continued) | in vivo direct epigenetic editing studies

	DBD	Effector domain	Target gene	Model/comments	Class[a]	Delivery	Ref.
Upregulation by DNA demethylation (continued)	dHFCas9	TET3	*Rasal1/Klotho*	Kidney fibrosis	IV	Lentivirus	230
	dCas9–SunTag	TET1	*H19/Igf2*	Silver–Russell syndrome	I & II	Plasmid-treated ES cells or mRNA-treated preimplantation embryos	186
	dCas9–SunTag	TET1	*Fgf21*	Metabolic syndrome	IV	Hydrodynamic tail-vein injection of plasmid	231
	dCas9–SunTag	TET1	*H19/Igf2*	Silver–Russell syndrome	I	piggyBac transposon-treated embryos	187
	dCpf1	TET1	8 ICRs	Parthenogenesis (allele-specific, multiplexing)	II	mRNA injection oocytes	193
	dCas9, ZF	TET2, TDG	*Cxcl11*	Asthma	IV	Protein delivery (intranasal and inhalation)	138
Repression/ splicing by histone modifications	ZF	G9A	*Fosb*	Cocaine use disorder	IV	Intracranial HSV	123
	ZF	G9A	*Cdk5*	Cocaine use disorder, depression	IV	Intracranial HSV	220
	ZF	Suvdel76	*Dlg4/PSD95*	Alzheimer disease, ageing	IV	Intracranial HSV	124
	ZF	G9A	*Fosb*	Depression	IV	Intracranial (Cre-dependent) HSV	217
	ZF	G9A	*Fosb*	Aggression	IV	Intracranial (Cre-dependent) HSV	212
	ZF	G9A	*Cdk5*	PTSD	IV	Intracranial HSV	219
	dCas9–KRAB	MCP–LSD1	Enhancers	Haematopoiesis	III	Xenografts	121
	dCas9	HDAC8	*Fos enhancer2*	Transcriptional bursting	IV	Intracranial lentivirus	213
	dCas9	HDAC3	*Dpp4*	Colon cancer/liver metastases	III	Orthotopic transplantation of transduced cells	208
	dCas9	Suv39	*Dux4*	FSHD	IV	Intramuscular AAV	222
	dCas9	SET2	*Srsf11*	Cocaine use disorder	IV	Intracranial plasmid	224
Activation by histone modifications	dCas9	p300	*Par4*	EMT in breast cancer	II	Orthotopic transplantation of transduced secondary tumour cells	207
	dCas9	p300	*Pdx1* (liver), *Enh2* (to check Fos levels)	CRISPR–p300-expressing mice — model development	I	Transgenic mice, research tool	144
	dCas9	p300	*B4galnt2*	Proof of concept — not sustained		LNP	55
	dCas9	p300	*Arc* SARE	Alcohol use disorder	IV	Intracranial lentivirus	221
	dCas9	p300	*Gad1*	Alzheimer disease	IV	Lentivirus	223

AAV, adeno-associated virus; DBD, DNA binding domain; dCas9, deactivated Cas9; DNMT3A, DNA methyltransferase 3A; DNMT3A3L, DNMT3A in combination with DNMT3L; EMT, epithelial–mesenchymal transition; ES cell, embryonic stem cell; FSHD, facioscapulohumeral muscular dystrophy; HDAC3, histone deacetylase 3; HSV, herpes simplex virus; ICR, imprinting control region; i.u., intrauterine; i.v., intravenous; LNP, lipid nanoparticle; NHP, non-human primates; PTSD, post-traumatic stress disorder; sgRNA, single guide RNA; TALE, transcription activator-like effector; ZF, zinc finger. [a]Refers to class I: transgeni c models; class II: epigenetically reprogrammed disease models; class III, therapeutic ex vivo treatments; class IV, therapeutic in vivo treatments.

embryo[197]. Although not exploited as animal disease models, these studies highlight the potential utility of early embryonic or developmental epigenetic editing to model disease in diverse species.

Neither transgenic nor early embryonic expression models are sufficient to prove therapeutic efficacy of epigenetic editing. To demonstrate true therapeutic efficacy of gene-targeted DNA or histone modifications, transient expression of the epigenetic editor must specifically and stably modify the epigenome, effectively regulate gene expression and rescue disease phenotype. To inform therapeutic promise of direct epigenetic editing, reviewed below are, first, transplantation studies of ex vivo epigenetically reprogrammed cells (denoted class III in Table 3) and, secondly, therapeutic effects of epigenetic editors delivered in vivo (class IV) (Fig. 3).

Class III studies

Given the challenge of achieving efficient and cell-type-specific delivery of large-sized catalytic epigenetic editors in vivo, reprogramming cells ex vivo by epigenetic editing followed by transplantation of the reprogrammed cells opens up new research and treatment avenues for certain diseases[105] (Table 3). For example, the transplantation of TALE–TET1 reprogrammed β-cells to induce β-cell proliferation is very promising for the treatment of diabetes[198], and alternative routes are now being

explored[199]. In addition, epigenetically edited HSPCs, when engrafted into the bone marrow of mice, demonstrated that the targeted DNA methylation was maintained during differentiation, allowing study of the effect of silencing p15 on haematopoiesis[140]. Most studies describing xenografting of direct epigenetically edited cells are in the fields of oncology and neurodegenerative diseases (Table 3), as described below.

In the field of oncology, disease models are frequently obtained by injecting animals with tumour cells, manipulated before xenografting

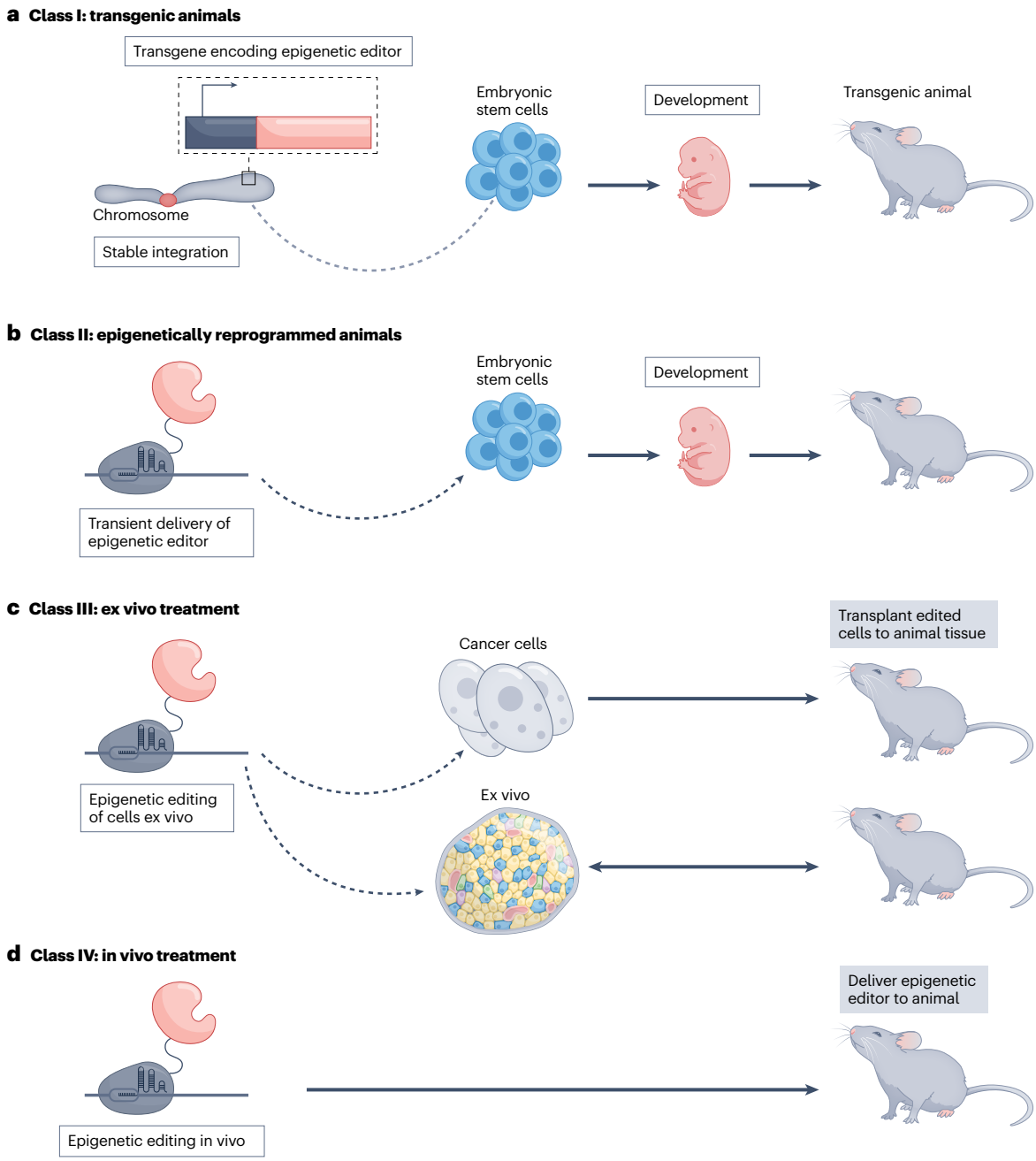

Fig. 3 | Types of epigenetic editing in vivo. a, Class I: embryonic stem cells are genetically modified to stably express a transgene encoding the desired epigenetic editor (fusion between DNA binding domain (DBD) (grey) and catalytic writer–eraser (red)). These cells then undergo development to create a transgenic animal in which each cell contains the DNA encoding the epigenetic editor. Epigenetic editing can take place during development or in the adult animal, sometimes in specific tissues, depending on how expression of the transgene is controlled temporally or spatially. **b**, Class II: the epigenetic editor is transiently expressed in embryonic stem cells or other early developmental stages to enable epigenetic editing of target genes. Effects of this editing are assessed later in development or in the adult animal. **c**, Class III: cancer cells, or cells isolated from an animal, undergo epigenetic editing ex vivo and are transplanted back into a particular tissue in the animal. **d**, Class IV: epigenetic editors are delivered in vivo in an animal disease model and target genomic regions associated with the disease.

to assess the functional effects of the intervention. Effects of editing DNA methylation have been shown in vivo using tumour cell lines engineered to stably express ZF–DNMT3A[167,200], TALE–TET1 (ref. 201) or dCas fusions[202–204]. Importantly, transient expression of CRISPRoff to silence *MGMT* expression, sensitized intracerebrally injected primary glioblastoma cells to temozolomide[205]. These studies each support the therapeutic relevance of direct epigenetic editing of a single cancer-associated gene (see Table 3) (alone or in combination with chemical drugs[206]), resulting in less-aggressive tumour characteristics.

The therapeutic potential of modulation of expression of a single cancer-related gene has also been shown by direct epigenetic editing of histone modifications. For example, injection of recurrent tumour cells stably expressing dCas9–p300 to activate *Par4* attenuated breast cancer tumour growth and improved survival[207]. Given that *Par4* epigenetic editing sensitized the tumour cells to chemotherapy, it is likely that even transient *Par4* activation would increase the efficacy of existing therapies. A study of colorectal cancer in mice applied lentiviral expression of dCas9–HDAC3 to reduce *Dpp4* promoter H3K27ac, *Dpp4* expression, tumour size and, importantly, mortality[208]. In these studies the epigenetic editors were stably expressed, therefore, future studies are needed to determine the persistence of epigenetic editing and long-term therapeutic potential.

Ex vivo transplantation studies have also explored epigenetic editing in neurodevelopmental disease, such as Rett syndrome and fragile X syndrome (FXS). These diseases are marked by epigenetic dysregulation of key developmental genes, namely, *MECP2* and *FMR1*, respectively, and preclinical studies show promise of direct epigenetic editing therein. An FXS mouse model phenotype, in which *Fmr1* is repressed, was rescued by transplantation of ex vivo induced pluripotent stem cells treated with dCas9–TET1 targeted to *Fmr1* (ref. 209). Two key features mark the translational importance of their earlier study using reporter mice[210]. First, direct epigenetic editing was sufficient to activate *Fmr1* expression in postmitotic neurons cultured from the FXS mouse model. This is noteworthy, because *FMR1* with repeat expansion is heterochromatic and transcriptionally silenced. Second, persistent lentiviral expression of dCas9–TET1 and sgRNAs in embryonic cells was sufficient for sustained *Fmr1* activation in neurons even up to 3 months after transplantation. Such persistent activation demonstrates that endogenous mechanisms of *Fmr1* repression did not override the epigenetic editing in the presence of dCas9–TET1. As the construct was stably expressed via lentiviral delivery, it is unclear whether transient epigenetic editing would yield similar effects. Future research is needed to establish maintenance of direct epigenetic editing of *Fmr1* in vivo using transient delivery methods. A complementary study applied epigenetic editing to human embryonic stem cells (ES cells) derived from patients with Rett syndrome, in which one allele of X-linked *MECP2* was mutated[211]. dCas9–TET1 targeted to wild-type *MECP2* on the inactivated X chromosome was sufficient to demethylate and activate *MECP2* expression and rescue deficits in human ES cell-derived neurons[211]. Although the latter study did not transplant the edited neurons to a mouse model of Rett syndrome, these studies underscore the enormous therapeutic potential of epigenetic editing in neurodevelopmental disease.

In summary, our understanding of epigenetic editing has grown with studies of transplantation of ex vivo reprogrammed cells and the generation of animal models of disease. Ex vivo reprogramming of naive cells, for example, for regenerative medicine, is another powerful application (reviewed elsewhere[105]), but the true potential of epigenetic editing lies in reprogramming somatic disease cells. Below, the therapeutic effects in various preclinical disease models are comprehensively summarized for direct epigenetic editors (class IV), and the sustainability of the effect if the epigenetic editor was delivered transiently is highlighted (Table 3).

Class IV studies

Editing histone modifications. Epigenetic editing in vivo was first shown by direct rewriting of histone modifications[123] and applied to studies of psychiatric disorders. Two highly pervasive psychiatric disorders, depression and addiction, reflect dysregulation of the medial corticolimbic circuit and are caused by an interaction between underlying epigenetic and/or genetic susceptibility and chronic exposure to stress or drugs. Pioneering preclinical studies have demonstrated in vivo direct epigenetic editing of the gene *Fosb*, which encodes a key transcription factor underlying addiction and depression. Transient expression of *Fosb*–ZF–G9a in the mouse brain blocked cocaine-induced *Fosb* expression and reward behaviour[123] and decreased aggression in mice[212]. The persistence of these behavioural effects is unknown, because all were measured during transient expression of the epigenetic editor.

Activity-induced, transient expression of immediate-early genes, such as *Fosb*, is a crucial mechanism of neuronal plasticity and is accompanied by transient acetylation of immediate-early gene promoters. Such genes, including *Arc*, *Fos*, *Fosb*, *Nr4a1* and *Npas4*, are prominent targets in the neuroepigenetic editing literature. Epigenetic editing of *Fos* with dCas9–HDAC8 in mouse hippocampus reduced the number of high-Fos-expressing hippocampal neurons[213]. A follow-up study targeted acetylation of the *Fos* enhancer using the dCas9–p300 transgenic mouse increased endogenous neuronal physiology and signalling[144]. To address the thousands of heterogeneous intermingled cell types in brain tissue[214–216], recent studies applied cell-type-specific expression of epigenetic editors in the mouse brain[217,218]. Neuronal-subtype-specific, transient expression of *Fosb*–ZF–G9a was sufficient to promote depressive-like behaviour for at least 4 days beyond *Fosb*–ZF–G9a epigenetic editor expression[217].

Activation of immediate-early genes in the brain leads to downstream activation of target genes essential to neuronal plasticity. One such target gene of *Fosb* is *Cdk5*, which encodes a neuron-specific kinase implicated in reward processing, stress and memory. Epigenetic editing using *Cdk5*–ZF–G9a defined the epigenetic regulation of *Cdk5* in mouse models of post-traumatic stress disorder (PTSD)[219], cocaine reward and stress[220]. Finally, a study of alcohol use disorder targeted dCas9–p300 to a key immediate-early gene enhancer, the *Arc* SARE[221]. dCas9–p300 increased *Arc* mRNA, *Arc* promoter H3K27Ac, *Arc* SARE enhancer RNA (eRNA), and the interaction strength between *Arc* SARE and *Arc* promoter, rescuing the behavioural effects of alcohol exposure[221].

Beyond psychiatric disorders, an early in vivo brain study applied targeting of SUV39H1 to repress *Psd95* expression via H3K9me3 enrichment[124]. Such repression was sufficient to regulate synapse and spine maturation of hippocampal neurons in vivo. FSHD is another key disease target for epigenetic editing, given that it is caused by epigenetic derepression of *DUX4*. Epigenetic editing with SUV39H1 SET domain to suppress *Dux4* rescued phenotypes in a mouse model of FSHD[222]. Finally, CRISPR–dCas9–p300 increased *B4galnt2* expression in hepatocytes[55] and neuronal *Gad1* expression in a mouse model of Alzheimer disease, ameliorating memory deficits[223].

These studies demonstrate the promise of modulation of the expression of a single gene by histone epigenetic editing to interfere

with disease pathogenesis. Although chromatin regulation of gene expression is well established, recent studies have found that certain histone modifications are also strongly predictive of alternative splicing. Epigenetic editing is uniquely suited to establish the functional role of the epigenome in alternative splicing. In mouse brain, expression of dCas9–SET2 to enrich H3K36me3 at the targeted site indeed drove alternative splicing of *Srsf11* and cocaine reward behaviour[224]. Importantly, epigenetic editing in these cases regulated alternative splicing without altering gene expression levels.

Editing DNA methylation. Various delivery methods have been applied to induce DNA demethylation or methylation editing in brain (see Table 3 for a comprehensive overview). Preclinical models of Alzheimer disease include lentiviral delivery of dCas9–TET1, resulting in derepression of cathepsin D to degrade amyloid-β (Aβ)[225] or dCas9–DNMT3a to silence *App* (encodes Aβ precursor protein)[226]. Intranasal administration of photo-inducible exosomes containing dCas9–DNMT3A ribonucleoprotein targeting *Bace1* (encodes β-secretase 1) reduced amyloid pathology and improved recognition memory impairment[227]. In preclinical studies of Parkinson disease, silencing of *SNCA* (encodes α-synuclein) with dCas9–DNMT3A using brain-targeted exosome delivery alleviated Parkinson disease-like impairments[228], and silencing of *Ttr* in the mouse prefrontal cortex by plasmid delivery of dCas9–DNMT3A3L alleviated depression-like behaviour[229].

The first demonstration of a therapeutic effect of direct DNA methylation editing beyond neurological disorders was the amelioration of kidney fibrosis by stable lentiviral expression of dCas9–TET3 to induce expression of *Rasal1* and *Klotho*[230]. In vivo maintenance of hit-and-run editing has also been assessed for the first time for DNA demethylation by transient expression of dCas9–SunTag–*Tet1*, targeted to *Fgf21* in mouse hepatocytes[231]. However, the effect upon hydrodynamic tail-vein injection of the all-in-one plasmid, was not maintained at day 14. As also described earlier, combinations of effector domains might improve maintenance of epigenetic reprogramming. Indeed, CRISPRoff (containing DNMT3A3L and KRAB) silenced *MGMT* expression upon transient mRNA electroporation in primary glioblastoma cells for at least 10 days upon transplantation[205]. Exciting recent data using this combination of three repressors (KRAB, DNMT3A and DNMT3L) as ZF fusions to silence *Pcsk9* demonstrate effective lowering of cholesterol levels for at least 1 year upon a single intravenous dose of LNP-mediated mRNA delivery (and at least 43 days for an all-in-one ZF fused to all three proteins)[26]. Importantly, the effect of this ZF–KRAB–DNMT3A3L silencing of *Pcsk9* was maintained even after forced liver regeneration for more than 2 months, with an efficiency comparable to that obtained through conventional gene knockout. The maintenance of induced silencing by DNA methylation of the *Pcsk9* locus has been independently confirmed up to 6 months upon transient expression of a bacterial DNA methyltransferase (MQ1) fused to TALE[78] (see Box 4). The follow-up humanized transgenic mouse and primate study not only validated the efficiency and durability of epigenetic editing, but also demonstrated reversibility of the induced silencing by targeting TET1 to the *PCSK9* locus[27]. Importantly, restoration of expression was maintained for at least 42 days in vivo. Beyond the liver, sustained activation of gene expression was recently demonstrated in the lungs by vector-free airway delivery of recombinant protein ZF or dCas9/gRNA fused to TET1 and TDG (thymine DNA glycosylase) targeting *Cxcl11* (ref. 138). Using a combination of nebulized inhalation and intranasal solution, *Cxcl11* was demethylated and derepressed in mouse lung epithelial cells, and mice gained responsiveness to interferon-γ (IFNγ).

In summary, the extant literature on direct epigenetic editing in vivo supports the enormous therapeutic promise of this approach, particularly for neurological, neurodegenerative and psychiatric diseases, for which early preclinical studies have shown promising results. However, these studies generally assessed outcomes while the editors were expressed, therefore only limited insights into epigenetic memory upon direct epigenetic editing in brain tissue is available. To our knowledge, there is only one in vivo study that has assessed long-term effects in the brain. In this study, AAV delivery of ZFs fused to the unmodified histone H3 tail (H3K4me0) and a non-catalytic DNA methyltransferase (DNMT3L) domain was sufficient to recruit endogenous DNA methyltransferases to repress prion protein expression and protect from prion-induced pathology[53]. As the AAV contained an auto-silencing promoter, it was possible to ascertain that this indirectly induced DNA methylation was persistent for at least 13 weeks. However, for dividing cells, the addition of KRAB was required to maintain repression. This observation fits the concept that, depending on the gene context, a combination of effector domains is likely needed to ensure robust sustained effects.

Clinical translation of epigenetic editing

At least 14 companies have been founded to translate epigenetic editing to the clinic (Table 2). Where disclosed, the clinical tools primarily apply targeted DNA methylation or demethylation to repress or activate pathological gene expression, respectively.

The first direct epigenetic editing tool that entered a clinical trial, namely OTX-2002 (Omega Therapeutics[10]), received FDA orphan drug designation in November 2022. OTX-2002 (ref. 137) induces silencing of *MYC*, an oncogene implicated in more than half of all human cancers. Specifically, OTX-2002 applies combinatorial epigenetic repression using *MYC*-targeted ZF–MQ1 (ref. 189) and ZF–KRAB to methylate both DNA and, indirectly, histones proximal to the *MYC* oncogene[137]. Unfortunately, evaluation of OTX-2002 was not continued beyond the phase I/II MYCHELANGELO I study that included 24 patients with hepatocellular carcinoma and other solid tumours.

Hepatitis B virus (HBV) is another key target of epigenetic therapies. Tune-401 (Tune Therapeutics), which has entered clinical trials in the Western Pacific Region, applies CRISPR-based DNA methylation to repress viral hepatitis genes (both those that are integrated into host DNA and those that are episomally maintained covalently closed circular DNA (cccDNA)) to address hepatitis B. In addition, the FDA has granted orphan drug designation status for EPI-321 (Epicrispr), which aims to restore DNA methylation to the *DUX4* locus in patients with FSHD and the first patient was treated in 2025. As Epicrispr uses dCasMINI (dCas12f)[160,161], its smaller size allows administration using AAV.

Several companies are investigating epigenetic editing approaches for the treatment of neurodegenerative diseases, such as Alzheimer disease (*APOEe4*), Duchenne muscular dystrophy (*UTRN*), amyotrophic lateral sclerosis (*C9orf72*) and FSHD (*DUX4*). Sangamo Biosciences is a pioneering company in the DNA-targeting space, having developed tools such as ZF–KRAB to repress mutant *HTT*[122] implicated in Huntington disease. SLS-004 (SEELOS Therapeutics) is dCas9–DNMT3A targeted to *SNCA*, which encodes α-synuclein implicated in Parkinson disease. Additional diseases and targets in the clinical space include neurodevelopmental diseases (*UBE3A*), chronic pain (*SCN9A*) and liver disease (*PSCK9*, *HNF4a*) (see Table 2 for references and details).

The rapid clinical translation of epigenetic editing has been fuelled by the success of the CRISPR–Cas9 genome editing platform, for which various clinical trials were initiated within a short time frame[41]. These trials include the first direct in vivo administration using LNP-formulated

Box 5 | Milestones in in vivo delivery of epigenetic editing tools

- **2014 — Herpes simplex virus**
  First in vivo epigenetic editing. Herpes simplex virus (HSV) transiently delivered zinc finger (ZF)–G9a to repress *Fosb* in a cocaine addiction mouse model, demonstrating robust locus-specific silencing via histone methylation[123].

- **2016 — In utero electroporation**
  In utero electroporation of an all-in-one CRISPR–dCas9–SunTag–*Tet1;Gfap*-single guide RNA (sgRNA) repressed *Gfap*–GFP reporter when delivered to the lateral ventricle of embryonic day 14.5 mouse embryos. This application is relevant to epigenetic dysregulation in developmental disorders, such as Angelman syndrome and fragile X syndrome[192].

- **2016 — Lentivirus**
  Lentivirus-delivered CRISPR–dCas9–*Tet1* to a transgenic mouse expressing a methylation-sensitive GFP reporter. GFP expression was activated by CRISPR–dCas9–*Tet1* injected into skin dermal cells and brain tissue. In 2018 this approach was applied in the brain to derepress *FMRP* in a mouse model of fragile X syndrome[210].

- **2020 — Hydrodynamic tail-vein injection**
  Hydrodynamic tail-vein injection of an all-in-one plasmid expressing CRISPR–dCas9–SunTag–*Tet1;Fgf21*-sgRNA transiently demethylated and activated *Fgf21* in mouse hepatocytes[231].

- **2021 — Cell-based delivery**
  Using ZF–KRAB, this study describes a promising platform to secrete constructs (easily transferable to the delivery of epigenetic editors). Implanted cells expressing ZF-based artificial transcription factors modulated gene expression in surrounding tissues[249].

- **2022 — Lipid nanoparticles**
  Lipid nanoparticles (LNPs) encapsulating either *B4galnt2*-sgRNA or synthetic mRNA for dCas9–VP64, dCas9–p300 or dCas9–VPH-SS18 (VP64–p65-HSF1–SWI/SNF) activated *B4galnt2* in liver cells in vivo. Separate encapsulation of sgRNA and dCas9 synthetic mRNA followed by injection of mixed LNPs resulted in twofold more efficient liver delivery than LNPs formulated with mixed dCas9 synthetic mRNA and sgRNA[55].

- **2024 — Exosomes (non-viral)**
  Intranasal delivery of dCas9–DNMT3A ribonucleoprotein targeted *Bace1*, reducing amyloid pathology in an Alzheimer disease mouse model[227].

- **2024 — Self-inactivating AAV**
  First use of an auto-silencing adeno-associated virus (AAV) vector. This enabled transient expression of ZFs fused to the unmodified histone H3 tail (H3K4me0) and a non-catalytic DNA methyltransferase (DNMT3L) domain (CHARM) recruiting endogenous DNMT3A to repress *Prnp* in a prion disease model, mitigating risks of long-term editor expression and circumventing AAV cargo size limitations for direct epigenetic editors[53].

- **2025 — Recombinant protein delivery**
  Recombinant protein epigenetic editor 'biologics' were developed for six ZF arrays or six CRISPR instances that recruited DNA demethylases, TDG and Tet, to *Cxcl11*. Intranasal and aerosol administration demethylated and derepressed *Cxcl11* in lung epithelial cells in mice[138].

- **2025 — LNPs: clinical translation**
  First demonstration of clinically relevant, durable epigenetic editing in primates. Tremblay et al.[27] used dCas-based epigenetic editors delivered via LNPs to target *PCSK9*, achieving long-lasting reduction of LDL cholesterol with robust efficacy and reversibility in non-human primates. This represents a major translational milestone.

mRNA to treat transthyretin amyloidosis[232] and resulted in the regulatory acceptance of the first CRISPR drug, Casgevy, to upregulate fetal haemoglobin expression via editing of a promoter DNA sequence[233]. The findings of the ongoing CRISPR–Cas9 clinical trials will address pressing questions of importance relevant to epigenetic editing, including efficacy and toxicity. In addition, given the observed chromosomal translocations and other abnormalities associated with on-target DNA editing[234], the need to optimize epigenetic editing as a non-invasive gene expression modulation approach might become urgent. Moreover, the two decades of clinical trials using zinc finger nucleases (ZFNs) or ZF ATFs set a precedent of safety, which might out-compete that of the DNA-intercalating CRISPR platform. Indeed, some epigenetic editing companies are exploiting ZFPs or TALEs (Table 2).

Delivery

Various approaches have been explored to achieve in vivo delivery of epigenetic editing tools (Box 5), including AAV and LNPs. Regarding delivery of CRISPR–Cas9 editing tools, AAV-mediated delivery is the most widely used approach in clinical trials[234], but severe adverse effects including deaths, potentially caused by immunological reactions to the viral capsid, clearly demand caution[235,236]. For example, a patient with Duchenne muscular dystrophy died after infusion with high-dose AAV9 expressing dSaCas9–VP64 (ref. 237) owing to systemic innate immune toxicity, potentially caused by the vector, but which might also be attributed to VP64 expression[238]. Moreover, long-term expression of the transgene when delivered using AAV might increase off-target effects; these effects can be mitigated by restricting expression of the epigenetic editor to a certain time window by using inducible systems (for example, hypoxia responsivity[239]) or Cas9 inhibitors[55,240]. In addition, AAV size constraints of the transgene (4.7 kb) hamper its utility for epigenetic editing, as often large effector domains need to be fused to the already large dCas9. Solutions aimed at addressing this limitation are being explored, including dual AAV vector technology based on reconstitution via mRNA trans-splicing[241] and smaller Cas variants[76,160,161,242]. Recently, an elegant study circumvented AAV cargo size limitations by delivering ZFs fused to the unmodified histone H3

tail (H3K4me0) and a non-catalytic DNA methyltransferase (DNMT3L) domain to recruit endogenous DNA methyltransferases[53].

Important studies have found that certain AAVs can effectively deliver Cas9 tools in many species, facilitating translational efforts[243], and improvements such as an all-in-one self-inactivating AAV vector[53,57,244] will certainly drive the field. In parallel, other delivery platforms are being exploited, including lentiviruses[245], which have a larger payload (~8–10 kb) and allow for tissue-specific all-in-one delivery[246]. Alternative non-viral delivery approaches are also being investigated[247,248], including engineered cells[249,250], scaffolds[251], virus-like particles[252] and recombinant proteins[138].

Following the success of LNP-mediated mRNA delivery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines, the use of LNPs to deliver RNA is receiving widespread attention and is being explored for genome editing in clinical trials[232]. This approach has been shown to be effective for the delivery of epigenetic editing tools[27,55,253] and was used in the clinical investigation of OTX-2002 (NCT05497453). Additional formulations, such as using two separate LNP formulations (mRNA encoding the dCas9 fusion versus the sgRNA) to prevent preferential encapsulation of guide over mRNA[55] might further increase efficacy. Additionally, incorporating targeting ligands to induce uptake of LNPs in preferred tissues can also assist in improving specificity[254], and its low immunogenicity allows repeated administrations of CRISPR–Cas9 without inducing immune issues[255]. Furthermore, at the level of sgRNA delivery, various approaches are being investigated to enhance its stability, distribution and cellular uptake, including chemical modifications and the use of chemically stabilized protecting oligonucleotides[256].

Conclusion and perspectives

Following the very first report of direct endogenous epigenetic editing modulating gene expression in 2003 (ref. 15), a decade passed before follow-up studies were published[18–25]. Over the past few years, the field has advanced significantly, with studies demonstrating target specificity, efficacy, context dependency, sustainability and preclinical therapeutic efficacy of epigenetic editing. Furthermore, epigenetic editing has emerged as a powerful hit-and-run approach for multiplexed and bidirectional expression modulation, without changing the genome sequence. Despite this progress, we have learned that the instructions for causality are not always simple, and we have begun to realize the rules of sustainability. In addition to enhancing our understanding of epigenetic reprogramming rules and improving delivery, identification of biomarkers to predict patient responsiveness to epigenetic editing might be needed to ensure effective clinical trials.

Beyond these fundamental considerations, further education about CRISPR[257] and the ethical implications of epigenetics[258,259] is essential for the progression of this technology[260,261]. Although epigenetic editing is considered less invasive than editing of DNA sequences and potentially easily reversible[27,52,75], both pose potential risks to human health and evolution. By comparison with editing genetic sequences, epigenetic editing is much less likely to be heritable, owing to erasure waves and epigenetic reprogramming during embryogenesis[262]. Although transgenerational inheritance of gene-targeted methylation and an associated phenotype has been shown in mice[263], this might be associated with the experimentally induced DNA scarring[264]. Rigorous and responsible innovation of epigenetic editing techniques should ensure the safe application of this powerful and versatile new therapeutic approach[265].

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

L.B. is a cofounder of Stylus Medicine and a member of its scientific advisory board, and has filed multiple provisional patents related to high-throughput characterization of transcriptional effector domains. The other authors declare no competing interests.

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