Molecular Therapy
Review

Epigenome editing based treatment: Progresses and challenges

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Epigenome editing is emerging as a transformative approach in clinical treatment, enabling precise modifications to gene expression without altering the underlying DNA sequence. The ongoing transition of epigenome editing techniques from foundational research to clinical applications highlights several key strategies. These include targeted DNA methylation/demethylation, histone modification, and transcriptional regulation. These approaches offer the potential for durable and reversible gene expression modulation, paving the way for precisely tailored therapies for genetic and complex diseases. Here, we review pioneering research, technological advancements, granted patents, and clinical trials that have been reported during the past decade. By synthesizing current research and development efforts, this review aims to provide insights into the promising landscape of epigenome editing and its potential to promote therapeutic interventions.

INTRODUCTION

In the past decade, targeted genome editing technologies have undergone remarkable advancements, solidifying their role as indispensable tools in the exploration of gene function and the treatment of various chronic and genetic disorders.[1–4] These groundbreaking methodologies have increasingly entered clinical trial phases, signaling a transformative shift in the field of genetics. A landmark moment in this evolution occurred in December 2023, when the US Food and Drug Administration (FDA) granted approval to Casgevy, the first CRISPR-based gene editing therapy developed by Vertex Pharmaceuticals and CRISPR Therapeutics for the treatment of sickle cell disease.[5,6] As we navigate these advancements in genome editing, we are also witnessing the emergence of innovative strategies for rectifying genetic abnormalities.[7,8] Recent studies have introduced powerful techniques for targeted DNA methylation/demethylation[9–13] and histone modification,[9,14–18] expanding therapeutic avenues beyond mere genetic alterations.

Epigenetic regulation plays a vital role in cellular processes, including expression regulation, chromatin stability, and differentiation.[19,20] This complex regulation hinges on two primary mechanisms: DNA methylation and histone modifications. DNA methylation involves the addition of a methyl group to the 5′ position of cytosine residues, predominantly at CpG dinucleotides within the mammalian genome. Typically associated with gene silencing, this modification can obstruct transcription factor (TF) binding and promote a compact chromatin structure.[21] Conversely, histone modifications can either activate or repress gene expression, contingent on the specific alterations made.[22] For instance, histone acetylation is generally indicative of active chromatin, whereas histone methylation's biological impacts are highly context dependent, varying with the specific site and degree of modification (mono-, di-, or tri-methylation). Notably, tri-methylation of histone H3 at lysines 9 and 27 (H3K9me3 and H3K27me3, respectively) is associated with transcriptional repression, while tri-methylation of H3 at lysine 4 (H3K4me3) is typically found at the promoters of actively transcribed genes.[23–25]

The intricate orchestration of DNA methylation is primarily governed by a family of enzymes known as DNA methyltransferases (DNMTs).[21] This family includes DNMT1, which preserves existing methylation patterns during DNA replication, and DNMT3A and DNMT3B, which establish new methylation marks in response to developmental and environmental signals. The ten-eleven translocation (TET) enzymes remove DNA methylation by oxidizing 5-methylcytosine, leading to the replacement of modified cytosines via the base excision repair pathway. Common histone methylation is primarily regulated by histone methyltransferases, which add methyl groups, and histone demethylases, which remove them. Histone acetylation, controlled by histone acetyltransferases, adds acetyl groups to lysine residues, while histone deacetylases (HDACs) remove them, resulting in a more compact chromatin structure linked to reduced transcriptional activity.[19] Dysregulation of these epigenetic modifiers has been implicated in a range of diseases, including cancers, neurological disorders, and metabolic syndromes. Therefore, strategically harnessing these enzymes for the targeted editing of epigenetic marks is vital for the development of novel therapeutics for challenging human diseases.

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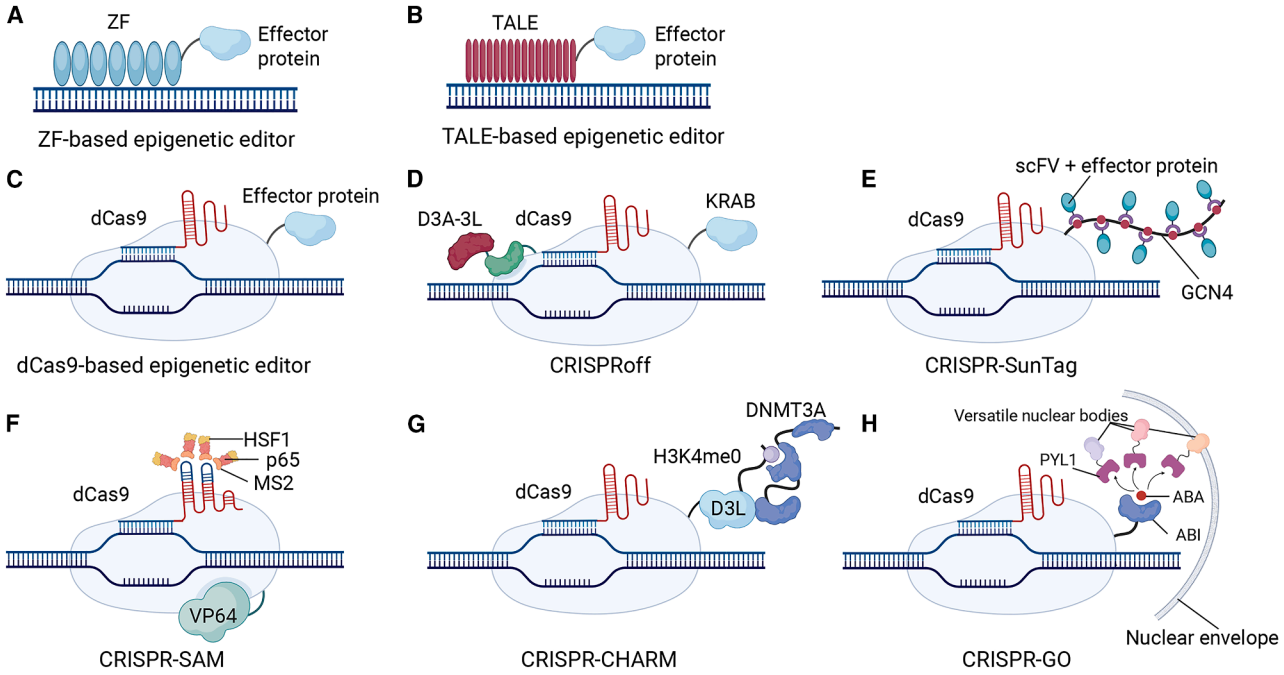

Figure 1. Schematic of ZF-, TALE-, CRISPR-based epigenome editors

Epigenetic editors fuse effector domains (DNA methyltransferases/demethylases, histone modifiers, transcriptional activators/repressors) to DNA-binding platforms (zinc finger [ZF], transcription activator-like effector [TALE], catalytically dead Cas9 [dCas9]) for locus-specific gene regulation. (A) ZF fusions for targeted epigenetic modification. (B) TALE fusions for epigenetic editing. (C) dCas9 fused to single effectors. (D) dCas9 fused with both DNMT3A-DNMT3L (D3A-3L) and the Krüppel-associated box (KRAB) domain to manipulate DNA methylation and H3K9me3. (E) dCas9-SunTag system, dCas9 fused to GCN4 (a peptide from a transcription factor protein in yeast) peptide arrays recruits multiple single-chain fragment variable (scFv)-effector fusions. (F) Synergistic activation mediator (SAM) system employed a triple activator combination of MS2-VP64 (a transcriptional activator composed of four tandem copies of VP16), transcription factor p65 (P65), and HSF1 (heat shock factor 1) to targeted sites to activate transcription. (G) CHARM system (an epigenetic silencer named coupled histone tail for auto-inhibition release of methyltransferase) activates the endogenous *DNMT3A* by coupling a histone H3K4me0 peptide to DNMT3L-dCas9, enabling DNA methylation at the target site. (H) CRISPR-GO (CRISPR-genome organization, a versatile system for targeting genome loci to nuclear compartments) enables spatial repositioning of genomic loci to specific subnuclear compartments (e.g., nucleoli) via dCas9-guided effector proteins. ABI, PYL1, and ABA, the components of chemical-inducible heterodimerization systems.

Recent advancements in targeted epigenome manipulation have been propelled by the integration of diverse DNA-binding platforms, such as zinc finger proteins (ZFPs),[26–28] transcription activator-like effectors (TALEs),[29,30] and the catalytically inactive CRISPR-associated dCas9 (endonuclease-dead Cas9) protein, with various epigenetic effects (EEs) (Figure 1).[31] These innovations have significantly enhanced the precision and feasibility of directed epigenetic modifications. Unlike permanent genetic changes, epigenetic alterations are often reversible and can be dynamically regulated, providing a flexible framework for controlling gene expression. This adaptability is particularly advantageous in therapeutic contexts, as fine-tuning gene expression can lead to safer and more effective interventions. The promise of this therapeutic reversibility has been concretely demonstrated through the specific engineering and application of diverse epigenome editors.

TALE AND CRISPR-DERIVED EPIGENOME EDITING TOOLS

TALE proteins achieve targeted DNA modification through modular DNA-binding motifs. Unlike ZFP that rely on multi-domain cooperativity, each TALE domain recognizes a single nucleotide via simplified interaction dynamics (Figures 1A and 1B). In a pioneering study, Joung and colleagues pioneered the development of TALE-based tools for targeted DNA demethylation by fusing engineered TALE proteins with the catalytic domain of the TET1.[30] Subsequent studies have built upon this foundation, exploring the use of TALE-DNMT and TALE-TET fusions to target and manipulate the methylation status of regulatory regions.[32,33]

Building upon the evolutionary CRISPR platform, researchers have developed advanced CRISPR-based epigenome editing tools. These tools typically utilize dCas9 coupled with various EEs to selectively modulate gene transcription without altering the underlying DNA sequence (Figure 1C). The first report regarding a CRISPR-based epigenetic tool involved fusing the original dCas9 with the transcription activation domain VP64 and the repression domain KRAB, known as CRISPRa and CRISPRi, respectively.[34] Subsequent studies have further optimized these systems by incorporating multiple EEs recruited to target regions, resulting in more pronounced effects on gene regulation. For instance, the dCas9-SunTag system (Figure 1E),

consists of a repetitive peptide array of 24 GCN4 molecules that can recruit multiple anti-GCN4 single-chain fragment variable (scFv) fusion effectors to target sites.[35,36] Meanwhile the synergistic activation mediator system employed a triple activator combination of MS2-VP64, P65, and HSF1(Figure 1F).[37] Early efforts in epigenome editing focused on manipulating single epigenetic marks. Initial studies targeted DNA methylation using enzymes such as DNMT3A, DNMT3L, and TET1 to direct methylation or demethylation at specific loci.[11–13,38–40] Similarly, parallel research explored editing of individual histone modifications by fusing enzymes such as LSD1, G9A, EZH2, DOT1L, and P300 to dCas9.[18,41] The field then evolved toward combination editing strategies, recognizing that concurrently targeting multiple epigenetic layers often yields more potent and persistent effects. Systems integrating distinct EEs have proven significantly more effective for stable gene regulation. A prime example is CRISPRoff (Figure 1D), which combines DNMT3A, DNMT3L, and the KRAB repressor domain to achieve markedly more durable and stable silencing than earlier single-component systems such as dCas9-KRAB or dCas9-DNMT3A-DNMT3L.[42,43] Conversely, the CRISPRon system combines the TET1 catalytic domain with engineered sgRNAs to precisely reverse CRISPRoff-induced methylation and restore gene expression.[42] However, emerging studies highlight that even these advanced combination tools, such as CRISPRoff, can exhibit context-dependent instability, with variable long-term effectiveness across different genomic loci.[44,45]

These findings collectively demonstrated that the durability and reversibility of epigenome editing tools vary significantly depending on their molecular design and genomic context. Such locus-specific variability currently limits their therapeutic reliability, necessitating customized optimization of editor design. Therapeutically, the dynamic reversibility of these tools offers a critical advantage over permanent genome editing. For example, CRISPRon's dCas9-TET1 fusion can erase DNA methylation marks imposed by CRISPRoff, enabling adjustable gene expression modulation.[42] A recent study demonstrated that sustained *PCSK9* silencing (over 100 days) achieved by epigenetic editors could be reversed using a dCas9-Tet demethylase system, which erased DNA methylation marks and re-activated the previously suppressed *PCSK9* expression.[46] This intrinsic adjustability could theoretically reduce long-term risks compared with permanent genome editing approaches, particularly for chronic disease management requiring adjustable therapeutic effects. However, the adjustability remains target-dependent, the genome-wide targeting of CRISPRoff results demonstrate that not all genes can be effectively silenced. Consequently, epigenetic editors exhibit varying editing efficacy in practical applications, necessitating further research to optimize their locus-specific performance.

Beyond these inherent biological constraints of efficacy and durability, significant translational hurdles related to delivery efficiency and immunogenicity further limit the practical application of CRISPR- and TALE-based epigenome editors. Recent published reviews have thoroughly examined the technology developments and progresses of CRISPR- or TALE-based targeted epigenome editing.[31,47] However, much of their translational utility is constrained by critical limitations in delivery efficiency and immunogenicity. The large size of full-length dCas9 (~4.2 kb) and TALE proteins (~3 kb per monomer) exceeds the cargo capacity of clinically proven delivery vectors such as adeno-associated viruses (AAVs) (limit: ~4.7 kb). In contrast, zinc finger (ZF) arrays (~0.5–1 kb) enable compact effector fusions (e.g., ZF-DNMT3A at 2.8 kb), allowing efficient packaging into viral vectors or lipid nanoparticles (LNPs). Furthermore, immunogenicity poses a significant barrier for CRISPR systems: pre-existing antibodies against *S. aureus* and *S. pyogenes* Cas9 are present in 78% and 58% of human donors, respectively,[48] whereas ZF scaffolds, derived from human transcription factors, exhibit markedly lower immune activation.[49,50] This is corroborated by clinical data showing that AAV-delivered ZFs are well tolerated at doses up to 5e13 vg/kg in humans,[51] although computational modeling and rational design were successfully applied to engineer CRISPR system to evade the immune response.[52] Collectively, these factors underscore why ZF-based platforms retain distinct advantages for *in vivo* epigenome editing, particularly in balancing deliverability, immunologic compatibility, and clinical feasibility—a progress explored in depth in the following section.

PROGRESSES OF ZF-BASED EPIGENOME EDITING *IN VIVO*

Current review selectively highlights studies utilizing ZF-based epigenome editing that were not comprehensively covered by previous reviews, particularly *in vivo* or pre-clinical breakthroughs. The foundations of targeted DNA methylation editing and gene repression date back to 1997, when Xu and Bestor first demonstrated targeted DNA methylation editing by fusing bacterial cytosine-5 methyltransferase *M.SssI* with ZFP arrays.[28] However, in the post-CRISPR era, attention to this platform has declined due to several significant challenges. These include the complex and often unreliable predictions of interactions between adjacent ZF modules, the labor-intensive process of constructing ZF arrays with multiple modules, and difficulties in navigating proprietary ZFP patents. Despite these challenges, key intrinsic features such as limited immunogenicity and a small packing size for delivery suggest potential therapeutic applications. Recent breakthroughs in ZFP-based *in vivo* epigenome editing have rekindled interest in this field, as discussed below.

To explore the potential treatment of hypercholesterolemia, a recent study on the mouse *Pcsk9* (proprotein convertase subtilisin/kexin type 9) gene,[53] which regulates the production of low-density lipoprotein (LDL) receptors, exemplifies the power of engineered transcriptional repressors (ETRs) in inducing long-lasting gene silencing *in vivo*. These ETRs, which include programmable DNA-binding domains (including dCas9, TALEs, and ZFPs) coupled with transcriptional repressor effector domains (e.g., Krüppel-associated box (KRAB), catalytic domain of DNMT3A (cdDNMT3A), and DNMT3L, were particularly effective in silencing the *Pcsk9* gene.

Table 1. Epigenome editing products from biotechnology companies and their developmental progress

Disease	Product	Status	Target disease/ indication	Target gene/ pathway	Vector	Editing method/platform	Note	Reference
Musculoskeletal	EPI-321	clinical	Facioscapulohumeral muscular dystrophy (FSHD)	*DUX4*	AAVrh74	GEMS (CRISPR based)	suppresses *DUX4* expression; improves muscle function in FSHD patient-derived myoblasts and humanized mice	Adhikari et al.[61]
	MDL-101	pre-clinical	LAMA2-congenital neuromuscular disease (CMD)	*LAMA1*	AAV9	CRISPR-GNDM	upregulates *LAMA1* to compensate *LAMA2* LoF mutations; improves muscle pathology and extend lifespan in mice and non-human primates	Qin et al.[62]; Therapeutics[63,64]
	MDL-202	pre-clinical	Myotonic dystrophy type 1	*DMPK*	AAV	CRISPR-GNDM	suppresses *DMPK*; restores mRNA splicing in muscle cells	Therapeutics[63,64]
	MDL-201	research	Duchenne muscular dystrophy (DMD)	*UTRN*	AAV	CRISPR-GNDM	reactivates *UTRN* to compensate for dysfunctional dystrophin in DMD cell	Therapeutics[63,64]
	MDL-103	research	Facioscapulohumeral muscular dystrophy (FSHD)	*DUX4*	AAV	CRISPR-GNDM	silencing toxic *DUX4*	Therapeutics[63,64]
Visual	EPI-141	research	Retinitis pigmentosa 4	*RHO*	AAV	GEMS (CRISPR based)	inhibits mutated *RHO* and produces normal RHO	Biotechnologies[65]
	EPI-111	research	Retinitis pigmentosa 11	*PRPF31*	N/A	GEMS (CRISPR based)	restores PRPF31 to normal physiological levels	Biotechnologies[65]
Cardiovascular	MDL-105	research	Dilated Cardiomyopathy	N/A	AAV	CRISPR-GNDM	N/A	Therapeutics[63,64]
Nervous system	MDL-104	research	Tauopathy (incl. Alzheimer's disease)	*MAPT*	AAV9	CRISPR-GNDM	suppresses Tau (MAPT) protein in humanized mouse models	Therapeutics[63,,64]
	MDL-206	research	Angelman syndrome	*UBE3A*	AAV9	CRISPR-GNDM	restoring paternal UBE3A levels in cortex	Therapeutics[63,64]
	MDL-207	research	Dravet syndrome	N/A	AAV	CRISPR-GNDM	N/A	Therapeutics[63,64]
Genetic	EPI-241	research	α1-antitrypsin deficiency	*SERPINA1*	AAV	GEMS (CRISPR based)	suppresses endogenous mutated *A1AT* expression	Biotechnologies[65]
	NT-Z001	pre-clinical	Inherited erythromelalgia chronic pain disorders	*NaV1.7*	AAV9	zinc finger based	addresses Nav 1.7 GoF mutation	Businesswire[66]
Oncological	OTX-2002	clinical	Hepatocellular carcinoma (HCC)	*MYC*	LNP	epigenomic controllers (mRNA)	suppresses *MYC* in human HCC mice models; downregulates 3 *MYC* in biopsies from NHP liver; enhances TKIs in treating HCC	Senapedis et al.[67]; Therapeutics[68]; Senapedis et al.[69]; Senapedis et al.[70]
	OTX-2101	pre-clinical	Non-small cell lung cancer (NSCLC)	*MYC*	LNP	epigenomic controllers (mRNA)	reduces *MYC* to inhibit tumor growth in NSCLC pre-clinical models	Therapeutics[68]; Eugine Lee et al.[71]
Metabolic	EPI-221	research	Heterozygous familial hypercholesterolemia (HeFH)	N/A	N/A	GEMS (CRISPR based)	N/A	Biotechnologies[65]

(*Continued on next page*)

Table 1. *Continued*

Disease	Product	Status	Target disease/ indication	Target gene/ pathway	Vector	Editing method/platform	Note	Reference
	N/A	pre-clinical	Hypercholesteremia	*PCSK9*	LNP	PCSK9-EE (CRISPR based)	durably suppresses the *PCSK9* and cholesterol levels in mice and non-human primates	Tremblay et al.[46]
	N/A	pre-clinical	Hypercholesteremia	*PCSK9*	LNP	TEMPO platform (CRISPR based)	durably suppresses *PCSK9* in hepatoma cells	Kwon[72]
Inflammatory	N/A	research	Inflammation	*CXCL1-8*	LNP	epigenomic controllers (mRNA)	N/A	Therapeutics[68]
Hepatic	N/A	research	Liver regeneration	*HNF4A*	LNP	epigenomic controllers (mRNA)	N/A	Therapeutics[68]
	TUNE-401	clinical	HBV	HBV DNA	LNP	TEMPO platform (CRISPR-based)	durable repression of HBV DNA and cccDNA	Cosgrove[73]
	CRMA-1001	pre-clinical	HBV	HBV DNA	LNP	HBV-EE (CRISPR-based)	durable suppression of viral DNA and HBsAg in treated animals, primary human hepatocytes infected with HBV	Anglero-Rodriguez[74,75]

AAV, adeno-associated virus; LNP, lipid nanoparticle; NHP, non-human primate; LoF, loss of function; GoF, gain of function; EE, epigenetic editor; CRISPR-GNDM, CRISPR-guided nucleosome modulation; GEMS, Gene Expression Modulation System.

Remarkably, ZF-based repressors were 5.7 and 2.8 times more potent than dCas9- and TALE-based counterparts. Genome-wide assessments revealed minimal off-target effects using RNA sequencing and whole-genome methylation sequencing. Additionally, a single intravenous dose of LNPs containing ZF-based repressors led to a significant reduction of PCSK9 in mouse plasma for up to 330 days. An evolved version of these repressors, called EvoETR, further improved specificity, reducing PCSK9 levels by about 70%, similar to CRISPR-Cas9, but without causing potentially harmful DNA breaks.

The *prp* gene (also known as the *PRNP* gene) encodes the prion protein (PrP), and alterations in this gene or its protein product can increase susceptibility to prion diseases.[54–56] Another breakthrough in epigenome editing involves using a compact silencer called CHARM (coupled histone tail for auto-inhibition release of methyltransferase),[57] which efficiently shut off the prion gene throughout the mouse brain when delivered through an AAV (Figure 1G). Although most of optimization of the CHARM effector is validated using the CRISPR-dCas9 platform, the brainwide long-lasting gene silencing experiments are performed using ZF as binding domain. A single intravenous injection could eliminate over 80% of the prion protein, well above the approximately 20% needed to improve symptoms. By coupling a histone H3K4me0 peptide to DNMT3L-dCas9, the CHARM system activates the endogenous DNMT3A methyltransferase, enabling robust and lasting DNA methylation at the target site. This approach utilizes the pre-existing but auto-inhibited DNMT3A, avoiding the need to deliver additional DNMT3A or similar molecules that may affect unintended genomic regions. By adapting an engineered AAV vector,[58] a 70%–90% decrease in *Prnp* transcripts and a 60%–80% reduction in PrP protein levels were observed.

Both innovative studies, leveraging the precision and programmability of ZFPs, hold great promise for developing effective therapies for certain genetic and neurological disorders. This renewed interest in ZF-based approaches is justified. One major advantage of ZF tools is their compact size, which allows for easier viral packaging,[27,51,59] especially with clinically used AAV vectors. Additionally, unlike Cas9, there are no known pre-existing antibodies against ZFs,[48,60] ensuring safer delivery. While CRISPR-based tools offer multiple targeting capabilities, such advantage declines once a single ZFP or TALE achieves the necessary epigenetic modifications. The benefits of ZFP-based epigenome editing provide a promising alternative to CRISPR, particularly for developing therapies targeting disorders with multiple copies of target loci.

PRE-CLINICAL APPLICATIONS FROM BIOTECHNOLOGICAL COMPANIES

Epigenome editing technology is emerging as a promising approach for disease intervention. A number of companies, including Chroma Medicine, Epic Bio, Tune Therapeutics, Omega Therapeutics, Moonwalk Biosciences, and Modalis Therapeutics, are focusing on developing advanced epigenome editing platforms and products aimed at precise, durable modifications on disease-related genes (Table 1). These companies primarily concentrate on targeted methylation mechanisms to selectively inhibit specific genes. The next section will explore several representative epigenome editing approaches (Figure 2) in detail.

Epi-silencing the *PCSK9* gene in treating atherosclerotic cardiovascular disease

LDL cholesterol is a key factor in atherosclerotic cardiovascular disease (ASCVD), making *PCSK9* a critical genetic target for hypercholesterolemia treatment. *PCSK9*-targeted drugs, such as

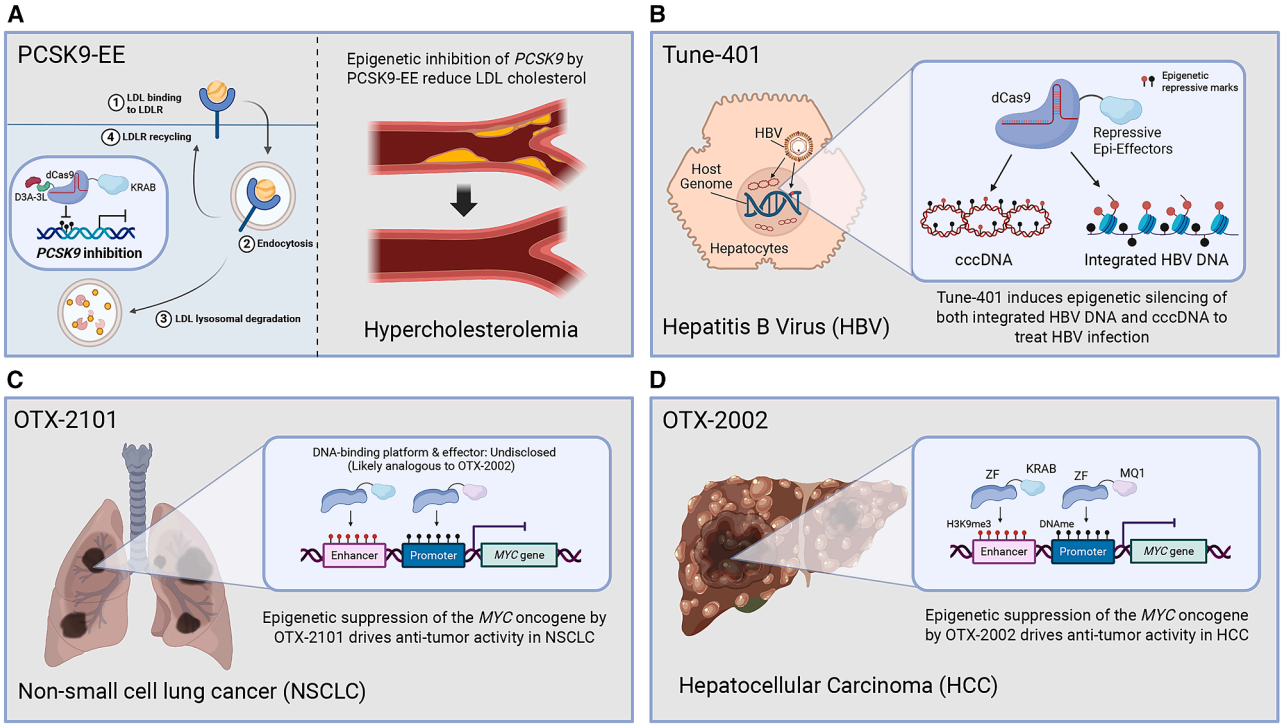

Figure 2. Representatives of epigenome editing products from biotechnology companies

Epigenome editing products from biotechnology companies include treatments for hypercholesterolemia, hepatitis B virus (HBV), and interventions for non-small cell lung cancer (NSCLC) and hepatocellular carcinoma (HCC). (A) *PCSK9*-targeted epigenetic editor (PCSK9-EE) represses *PCSK9* expression via DNA methylation, reducing LDL cholesterol in hypercholesterolemia. (B) Tune-401 induces epigenetic silencing of integrated HBV DNA and covalently closed circular DNA (cccDNA). (C) OTX-2101 silences the *MYC* oncogene, demonstrating anti-tumor efficacy in NSCLC models. (D) OTX-2002 epigenetically repress *MYC*, showing potent anti-tumor activity in HCC models.

FDA-approved alirocumab (Praluent) and evolocumab (Repatha), effectively lower LDL cholesterol and reduce ASCVD risk.[76] Recent advances using genome editing had achieved long-term reductions in LDL cholesterol by knocking out *PCSK9* gene *in vivo*. Base editors targeting *PCSK9* achieved a 90% reduction in PCSK9 and 60% lowering of LDL levels in living cynomolgus monkeys (*Macaca fascicularis*), with effects sustained for at least 8 months.[77] Conventional Cas9-mediated *Pcsk9* knockout in non-human primates (NHPs) produced up to 83% PCSK9 suppression and 77% LDL reduction, maintaining stability for over 3 years.[78] Nevertheless, to introduce reversible manipulations, at the 26th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT), two biotechnology companies showcased epigenome editing strategies for regulating *PCSK9* expression (Figure 2A). One demonstrated an epigenetic editor that led to an 80% reduction in circulating PCSK9 and a 58% drop in LDL cholesterol, sustained for up to 10 months in human transgenic mice.[53] Their latest study demonstrates the therapeutic potential of long-durable and reversible epigenetic editing *in vivo* for at least 1 year.[46] The other one reported its TEMPO epi-repressor platform resulting in a 75% decrease in circulating PCSK9 and a 56% reduction in LDL cholesterol over 4 months, with targeted DNA methylation marks maintained for at least 85 days. These advancements indicate strong potential for developing durable therapies for managing hypercholesterolemia and reducing ASCVD risk.

Epi-silencing covalently closed circular DNA in treating chronic hepatitis B virus infection

Hepatitis B virus (HBV) infection is a persistent and incurable condition that affects nearly 300 million people globally, significantly increasing the risk of liver cirrhosis and hepatocellular carcinoma (HCC). The virus integrates into the host genome and maintains extrachromosomal covalently closed circular DNA (cccDNA) reservoirs, which traditional treatments struggle to eliminate.[79] Initial clinical assessment of Cas9-mediated HBV therapy demonstrated only modest efficacy, reducing serum HBsAg levels by approximately 25%.[80] This limited response was further elucidated in a humanized FRG (*Fah*, *Rag*, and *Ilrg* knocked out) model with robust liver chimerism, where AAV-delivered Cas9 monotherapy induced transient suppression followed by complete viral rebound to baseline within 4 weeks. Critically, this monotherapeutic approach proved markedly inferior to combinatorial intervention pairing AAV-Cas9 with the nucleoside analog entecavir.[81] These limited efficacies likely reflect HBV's high mutation rate, which drives rapid evolution of CRISPR-resistant variants via mutations in gRNA-targeted regions of cccDNA. Epigenome editing has emerged as a promising

alternative, allowing for durable silencing of viral gene expression from both integrated HBV genomes and cccDNA without the risks associated with traditional genome editing.

At the 2023 HBV International Meeting, Chroma Medicine presented data showing that their epigenetic repressors significantly reduced hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg) levels *in vivo*, with 83% of their transgenic mice showing undetectable HBV DNA and HBsAg. Similarly, Tune Therapeutics showcased their TEMPO epi-repressor platform, which achieved consistent viral DNA silencing for over 550 days in mice with primary human hepatocytes transplanted from humans (Figure 2B). While further evaluation in NHPs is needed, the demonstrated efficacy and durability of these approaches suggest significant therapeutic promise against persistent HBV infection.

Epi-silencing *MYC* to treat epidermal growth factor receptor inhibitor-resistant NSCLC

Activating mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain lead to the receptor's constitutive activation, driving aberrant cell proliferation in patients with non-small cell lung cancer (NSCLC). Targeted EGFR tyrosine kinase inhibitors (TKIs) have transformed NSCLC treatment, achieving a 60%–70% response rate for patients with EGFR-activating mutations. Nevertheless, many patients with the T790M mutation still relapsed after TKI treatment.[82,83] Even with the third-generation TKI osimertinib to specifically target T790M, resistance still developed through various mechanisms, including new EGFR mutations (e.g., C797, G796, G724), bypass signaling pathways, epithelial-to-mesenchymal transition (EMT), oncogenic fusions, and cell-cycle gene changes.[83]

c-MYC is a key proto-oncogene and studies show that targeting *c-MYC* can overcome resistance to EGFR TKIs such as osimertinib in NSCLC patients. At the 2024 American Association for Cancer Research Meeting, a globally recognized conference focused on advancing cancer research and their clinical translation, Omega Therapeutics introduced a programmable epigenome editor, the MYC epigenomic controller (NSCLC MYC-EC), designed to selectively downregulate *MYC* expression (Figure 2C). This approach synergistically inhibits the growth of *EGFR*-T790M mutant NSCLC cells when combined with osimertinib. Notably, NSCLC cells resistant to osimertinib due to the *EGFR*-C797 mutation or EMT remain sensitive to *MYC* downregulation, offering a promising combination therapy for advanced *EGFR*-mutated NSCLC patients.

Epigenetic silencing of MYC for HCC treatment

HCC is one of the most common primary liver cancers in adults, predominantly driven by the overexpression of the *c-MYC* oncogene, which plays a critical role in HCC tumorigenesis.[84] Targeted inhibition of *c-MYC* induces tumor regression, positioning *c-MYC* as a key therapeutic target.[85–87] However, no *c-MYC*-targeted therapies are currently available for HCC.[88] The MYC protein lacks a structured binding pocket, which complicates the development of direct small-molecule inhibitors. Targeting *MYC* with small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) has emerged as a promising therapeutic strategy. Studies demonstrate that *MYC* siRNA achieves sustained *MYC* suppression for up to 8 days post-transfection[89] and inhibits HCC tumor proliferation over a 168-h (7-day) period.[90] Furthermore, weekly administration of *MYC* ASO reduced *MYC* expression by 15-fold in HCC and 22-fold in renal cell carcinoma tumor models, while suppressing *MYC*-driven tumorigenesis during the 4-week treatment period.[91]

Epigenome editing offers an alternative precise and durable way to downregulate *MYC* expression. A notable development is OTX-2002 (Figure 2D), an epigenetic therapy from Omega Therapeutics.[67] OTX-2002 utilizes a bicistronic mRNA to induce epigenetic changes at the *MYC* gene, leading to sustained suppression of *MYC* expression. In pre-clinical HCC models, OTX-2002 rapidly reduced MYC mRNA and protein levels, with effects lasting up to 15 days. Although OTX-2002 currently shows no significant advantage over siRNA and ASO in terms of durability, its epigenome editing mechanism provides greater flexibility and programmability. By engineering effector proteins (e.g., fusing more stable structural domains or incorporating targeting-enhancing modules), it holds potential to achieve longer-lasting therapeutic effects in future iterations. *In vivo* studies showed significant tumor reduction in both xenograft and orthotopic models, with good tolerability in mice. Furthermore, OTX-2002 enhanced the efficacy of TKIs such as sorafenib and lenvatinib, suggesting that it could reduce clinical doses and side effects while improving outcomes. Currently, OTX-2002 is in a phase 1/2 clinical trial (NCT05497453), offering a novel approach to overcome the limitations of existing *MYC*-targeting therapies for HCC.

Epigenome regulation in inheritable diseases

Epigenome regulation shows promise in addressing various genetic conditions, such as facioscapulohumeral muscular dystrophy (FSHD), the most common adult muscular dystrophy, affecting about 4.6 individuals per 100,000 globally. Researchers are exploring targeted epigenetic repression of the overexpressed *DUX4* gene as a potential therapeutic approach.[92,93] This has been achieved using dCas9-KRAB or other dCas9 repressor systems, including the SUV39H1 SET domain, MeCP2 TRD, HP1α, or HP1γ. Recently, Epic Bio announced plans to start a phase 1/2 clinical study to repress *DUX4* expression and reduce muscle cell death in FSHD, utilizing a muscle tissue-specific AAV vector (AAVrh74) for delivery.

Another promising application of epigenome editing targets the *SCN9A* (NaV1.7) gene, a key regulator of pain signaling.[94] Targeted repression of *SCN9A* is being investigated for treating various chronic pain conditions, including chemotherapy-induced peripheral neuropathy, as well as neurological and ophthalmic diseases.[95,96] These advancements highlight the potential of epigenome regulation in developing innovative therapies for challenging genetic conditions.[97]

While epigenome editing technologies in pre-clinical applications demonstrate promising therapeutic potential, their long-term translation hinges on robust intellectual property strategies. Biotechnology companies are actively securing patents not only for innovations nearing pre-clinical validation but also for early-stage techniques that remain distant from practical application. This proactive patenting serves a dual purpose: it safeguards future pipeline development by ensuring exclusivity in high-value targets (e.g., *PCSK9* for cardiovascular diseases or *MYC* in oncology) and creates strategic advantages in partnerships, funding, and commercialization. Importantly, these patents often reflect confidence in the platform's versatility, even when specific applications require further refinement. The following Patent Overview section expands on this interplay, offering a picture of how intellectual property landscapes both mirror current pre-clinical priorities and anticipate emerging directions in the field.

PATENT OVERVIEW

The pharmaceutical and biotechnology industries are launching animals (e.g., mice, rats, and monkeys) and pre-clinical studies to explore targeted epigenetic modulation for a variety of medical conditions, including chronic diseases, cancers, infectious diseases, and pain disorders (Table 2). Currently, many potential applications from companies specializing in epigenome editing are in the early stages of development, with only limited products undergoing clinical trials. To better understand their future strategic directions, we conducted a search of patent information from these leading companies using the PATENTSCOPE, an open-access database managed by the World Intellectual Property Organization (https://www.wipo.int/en/web/patentscope), and Google Patents (https://patents.google.com). Our findings, the details summarized in Table 3, indicate that many of these patents explore innovative approaches for treating not only common conditions but also rare genetic disorders and immune diseases (Figure 3). Representative diseases with available information are shown below.

Rett syndrome

recent patents WO/2024/163683 and WO/2023/010135 have been filed for using the dCas9-TET1 editing tool to regulate *MeCP2* gene expression. By designing multiple sgRNAs targeting the promoter region and optimizing an 80-amino acid linker in the dCas9-TET1 fusion protein, editing efficiency has been significantly enhanced. The latest patent (filed in 2024) also explores a ZFN-TET1 fusion protein with smaller size, providing potential for *in vivo* delivery and treatment options.

Friedreich's ataxia

to activate the silenced *FXN* gene caused by GAA expansion in its intron, patents WO/2023/010133 and WO/2024/163678 utilize dCas9-VP64 fusion proteins to enhance gene expression in induced pluripotent stem cells (iPSCs) with expanded *FXN* alleles. The 2024 patent also explores a ZFN-VP64, a smaller version of fusion protein, again indicating future *in vivo* applications.

Immune system regulation

epigenome editing can effectively modulate immune responses. Tune Therapeutics has several patents (WO/2024/064642, WO/2023/137472, WO/2023/137471) focused on regulating T cell-related genes. Chroma Medicine has filed patents targeting genes such as *CD274* and *TRAC*, while Modalis Therapeutics' patent (WO/2018/212361) addresses the *MYD88* pathway for treating related inflammatory and hematological conditions.

Other areas

companies are exploring various applications, including blood lipid regulation through *PCSK9* inhibition, with patents filed by Tune Therapeutics, Epigenic Therapeutics, and Omega Therapeutics. Omega Therapeutics is also pursuing a patent (WO/2023/283359) for the *SFRP1* gene to treat hair loss, and Navega Therapeutics is seeking a patent for genes associated with neurological diseases and chronic pain.

THERAPEUTIC POTENTIAL OF NON-CODING RNAs IN EPIGENETIC REGULATION

The dysregulation of non-coding RNAs (ncRNAs) represents a compelling therapeutic target, as these molecules function as master regulators of the epigenetic machinery governing DNA methylation, histone modifications, chromatin remodeling, and ultimately, gene expression. Distinct ncRNA classes exert precise control over specific epigenetic writers, erasers, and readers, making them potent candidates for restoring dysregulated epigenetic states in disease.

Long non-coding RNAs orchestrate targeted epigenetic silencing complexes. For instance, *XIST* directs X chromosome inactivation by recruiting PRC2 (catalyzing repressive H3K27me3 marks), while *HOTAIR* coordinates dual recruitment of PRC2 and LSD1 (enabling simultaneous H3K27me3 deposition and H3K4me2 demethylation) for precise gene silencing.[122] MicroRNAs (miRNAs) indirectly modulate the epigenetic landscape by targeting key enzymatic regulators: miR-29 induces global DNA hypomethylation via suppression of *de novo* methyltransferases DNMT3A/3B; miR-148a directly targets *DNMT3B* mRNA; and miR-101 inhibits *EZH2* (the catalytic core of PRC2), thereby reducing H3K27me3.[123–126] Circular RNAs, exemplified by *FECR1*, dynamically balance DNA methylation states by recruiting the demethylase TET1 while suppressing DNMT1 methyltransferase activity.[127] PIWI-interacting RNAs guide PIWI proteins to establish sequence-specific *de novo* DNA methylation, crucial for germline development and genomic stability.[128] Critically, aberrant expression or function of these ncRNAs is mechanistically linked to the pathogenesis across cancers, neurodegenerative disorders, and developmental diseases. This direct causative role, combined with their ability to programmatically control epigenetic effectors, positions ncRNAs as precision targets for developing epigenetic therapies aimed at resetting pathological gene expression programs.

It should be noted that most ncRNAs involved in epigenetic remodeling lack intrinsic targeting specificity to the pathogenic gene,

Table 2. Disease-associated epigenome editing applications *in vivo*

Disease type	Related diseases	Vector type	Target gene	Target organisms	Target tissue	Editing tool	*In vivo* delivery method	Reference
Metabolic diseases	hypercholesterolemia	LNP	*Pcsk9*	mice	liver	ZFP-ETR	tail vein injection	Cappelluti et al.[53]
	hypercholesterolemia	AAV8	*Pcsk9*	mice	liver	dCas9-KRAB	tail vein injection	Thakore et al.[98]
	hypercholesterolemia	LNP	*Pcsk9*	cynomolgus monkeys	liver	dCas9-DNMT3A3L + KRAB	intravenous infusion	Tremblay et al.[46]
	hypercholesterolemia	AAV2/8	*Pcsk9*	mice	liver	dNovaIscB-DNMT3A3L-KRAB	intravitreal injection	Kannan et al.[99]
	obesity	rAAV	*Mc4r*	mice	hypothalamus	dCas9-VP64	stereotaxic injection	Matharu et al.[100]
	type 1 diabetes	AAV2/9	*Pdx1*	mice	liver	dCas9-MS2-p65-HSF1	tail vein injection	Liao et al.[101]
Neurological and psychiatric disorders	alcohol use disorder	lentivirus	*Arc*	Sprague-Dawley rats	central nucleus of the amygdala	dCas9-P300 dCas9-KRAB	stereotaxic injection	Bohnsack et al.[102]
	Alzheimer's disease	rAAV	*Mapt*	mice	hippocampus	ZFP-KRAB	stereotaxic injection	Wegmann et al.[103]
	Alzheimer's disease	lentivirus	*amyloid precursor protein* (*App*)	mice	dentate gyrus	dCas9-DNMT3A	stereotaxic injection	Park et al.[104]
	Alzheimer's disease	lentivirus	*Ctsd*	mice	dentate gyrus	dCas9-TET1	microinjection	Park et al.[105]
	age-related memory impairment	lentivirus	*Per1*	mice	hippocampus	MS2:P65:HSF1 dCas9-VP64	stereotaxic injection	Kwapis et al.[106]
	autism spectrum disorder (ASD)	plasmid	*Mecp2*	mice	murine zygote	dCas9-DNMT3A3L	zygote microinjection	Lu et al.[107]
	autism spectrum disorder (ASD)	AAV9	*Mecp2*	mice	hippocampus	dCas9-DNMT3A	stereotaxic injection	Lu et al.[107]
	cocaine addiction	plasmid	*Nr4a1*	mice	nucleus accumbens (NAc)	dCas9-KRAB dCas9-VP64	stereotaxic injection	Carpenter et al.[108]
	transcallosal dysconnectivity	plasmid	*Sema6a*	mice	brain cortex	dCas9-SunTag-C11orf46	*in utero* electroporation	Peter et al.[109]
	Dravet syndrome (DS)	AAV9	*Scn1a*	mice	cerebral cortex	dCas9-VP64	intracerebroventricular injection	Colasante et al.[110]
	chronic pain	AAV9	*NaV1.7*	mice	dorsal root ganglion	dCas9-KRAB ZFP-KRAB	intrathecal injection	Moreno et al.[111]
	Huntington's disease	AAV6	*Htt*	mice	striatum	ZFP-KRAB	stereotaxic injection	Zeitler et al.[112]
	Parkinson's disease	engineered exosome	*Snca*	mice	brain	dCas9-DNMT3A	microbubbles injection with focused ultrasound	Kong et al.[113]
	tauopathy	lentivirus	*Gad1*	mice	prefrontal cortex	dCas9-p300	stereotaxic injection	Wan et al.[114]
	prion disease	rAAV	Prion gene	mice	brain	ZFP-CHARM CRISPR-CHARM	intravenous injection	Neumann et al.[57]
Muscular and skeletal disorders	disc degeneration	lentivirus	*Tnfr1*	Sprague-Dawley rats	intervertebral disc	dCas9-KRAB	intradiscal injection	Stover et al.[115]
	muscular dystrophy type 1A (MDC1A)	AAV9	*Lama2*	mice	muscle	dCas9-VP64	tail vein injection	Kemaladewi et al.[116]
	Duchenne muscular dystrophy (DMD)	AAV2/9	*Klotho*, *Utropin*	mice	muscle	dCas9-MS2-p65-HSF1	facial vein injection intramuscular injection	Liao et al.[101]

(*Continued on next page*)

Table 2. *Continued*

Disease type	Related diseases	Vector type	Target gene	Target organisms	Target tissue	Editing tool	*In vivo* delivery method	Reference
Ophthalmic diseases	inherited retinal dystrophies (IRDs)	rAAV	*Opn1mw*	mice	retina	dCas9-VPR	subretinal injection	Bohm et al.[117]
	retinitis pigmentosa	AAV2	*Nrl*	mice	retina	dCas9-VP64	subretinal injection	Moreno et al.[118]
Infectious diseases	HBV	plasmid	HBV viral surface open reading frame	mice	liver	TALEs-KRAB	hydrodynamic injection	Bloom et al.[119]
Renal diseases	acute kidney injury	AAV2/9	*Klotho*, *IL-10*	mice	kidney	dCas9-MS2-p65-HSF1	tail vein injection	Liao et al.[101]
	renal fibrosis	lentivirus	*Rasal1*, *Klotho*	mice	kidney	dCas9-TET3	renal artery/vein injection retrograde ureteral infusion intraparenchymal injection	Xu et al.[120]
Psychiatric disease	depression	AAV	*Ttr*	mice	prefrontal cortex	dCas9-DNMT3A3L	stereotaxic surgery	Stein et al.[121]

similar to broad-acting epigenetic enzyme inhibitors, such as HDAC inhibitors, and DNMT1 inhibitors, such as azacytidine and decitabine. Nevertheless, these ncRNAs could be co-delivered with ZF-, TALE-, or dCas9-based systems to enhance the editing efficiency and durability. For example, combining dCas9-Tet1 with azacytidine synergistically increased *MyoD* induction and reprogramming efficiency in fibroblast-to-myocyte conversion. This demonstrates the potential of a synergetic effect.[11] Therefore, combined treatments that pair ncRNAs with targeted epigenome editors through endogenous DNMTs recruitment, such as CHARM, represent a promising strategy that warrants further exploration for human disease applications.

CHALLENGES AND FUTURE PERSPECTIVES

The past decade has witnessed remarkable expansion in the field of epigenome editing, characterized by a substantial increase in fundamental research and proof-of-concept studies that have laid the groundwork for promising therapeutic applications. However, the transition from laboratory research to clinical practice is fraught with substantial technical and translational challenges. One of the key challenges is the development of robust delivery systems capable of effectively targeting and modulating the epigenome *in vivo*. Additionally, concerns surrounding the specificity and safety profiles of current epigenome editing techniques remain prominent. Our understanding of the complex epigenomic landscape and how to navigate its intricacies is still incomplete. Addressing these challenges will require concerted interdisciplinary efforts before the full clinical potential of epigenome editing can be realized. In the following sections, we review the major concerns related to refining epigenome editing (Figure 4) and propose potential solutions that are emerging.

Non-specific editing from EEs

The advent of the ZFNs, TALENs, and CRISPR systems has revolutionized genome cleavage capabilities, extending applications beyond merely DNA sequence. Most current epigenome editing tools employ catalytically inactive versions of DNA-binding proteins in conjunction with one or more EEs. While these advancements enable researchers to probe the causal relationships between epigenetic marks and transcriptional outcomes, a pressing challenge remains: the need for a comprehensive assessment of the global on-target and off-target activities of these editing tools (Figure 4A). Ensuring precise and specific targeting is essential for deriving reliable conclusions regarding epigenetic mechanisms.

Concerns regarding off-target effects in genome editing stem from loci that possess similar sequences to the desired on-targets. Experimental results obtained from protein-DNA conformation, genome-wide deep sequencing, and global binding profiling concluded that mismatches or gaps outside the seed region of sgRNA binding sites posed limited impact on its non-specific anchoring.[37,129–132] However, the uneven distribution of the pre-deposited DNA methylation and the activity of endogenous DNMTs complicate the accurate evaluation of epigenome editing. Current literature provides preliminary insights into the general

Table 3. Patent portfolio by epigenome editing companies

Company	Title	Patent no.	Target genes	Diseases/applications
Tune Therapeutics	systems, compositions, and methods for modulating expression of Methyl-CpG Binding Protein 2 (*MECP2*) nd X-Inactive Specific Transcript (*XIST*)	WO2024163683	*MECP2*, *XIST*	Rett syndrome
	compositions and methods for modulating expression of Methyl-CpG Binding Protein 2 (*MECP2*)	WO2023010135	*MECP2*	Rett syndrome
	compositions and methods for modulating expression of Frataxin (*FXN*)	WO2023010133	*FXN*	Friedreich's ataxia (FA)
	fusion proteins and systems for targeted activation of Frataxin (*FXN*) and related methods	WO2024163678	*FXN*	Friedreich's ataxia (FA)
	compositions, systems, and methods for regulation of hepatitis B virus through targeted gene repression	WO2024040254	HBV genome	hepatitis B virus (HBV)
	compositions, systems, and methods for modulating T cell function	WO2024064642	genes influence T cell functions	regulate T cell functions
	compositions, systems, and methods for programming T cell phenotypes through targeted gene repression	WO2023137472	genes influence T cell functions (repression)	regulate T cell functions
	compositions, systems, and methods for programming T cell phenotypes through targeted gene activation	WO2023137471	genes influence T cell functions (activation)	regulate T cell functions
	compositions, systems, and methods for targeted transcriptional activation	WO2024015881	*FXN*, *IL-2*, *CCR7*	develop epigenetic activation tools
	compositions, systems, and methods for reducing low-density lipoprotein through targeted gene repression	WO2023250511	genes or regulatory element thereof that regulate LDL	hypercholesterolemia
	compositions and methods for multiplexed activation and repression of T cell gene expression	WO2025029840	gene-influenced T cell functions	regulate T cell functions
	compositions, systems, and methods for lymphoid cell differentiation using targeted gene activation	WO2025038494	lymphoid cell differentiation-related genes	regulate lymphoid cell differentiation
	compositions and methods for modulating *IL-2* gene expression	WO2025029835	*IL-2*	regulate T cell functions
	epigenetic editing methods and systems for differentiating stem cells	WO2025059073	stem cell differentiation-related genes	regulate stem cell differentiation
Chroma Medicine	fusion proteins for epigenetic regulation	WO2024220857	*PCSK9*, *CLTA*	develop epigenetic regulation tools
	compositions and methods for epigenetic regulation of *PCSK9* expression	WO2024186896	*PCSK9*	hypercholesterolemia
	compositions and methods for epigenetic regulation of complement factor expression	WO2024178402	complement factor-encoding genes	complement dysfunction and complement overactivity-related diseases
	compositions and methods for epigenetic regulation of *ANGPTL3* expression	WO2024145615	*ANGPTL3*	heart disease
	compositions and methods for epigenetic regulation of HBV gene expression	WO2024064910	HBV genome	hepatitis B virus (HBV)
	compositions and methods for epigenetic regulation of *CD247* expression	WO2024081879	*CD247*	genetically engineering immune cells
	compositions and methods for epigenetic editing	WO2023250148	*PCSK9*	develop epigenetic regulation tools
	compositions and methods for epigenetic regulation of *TRAC* expression	WO2023250490	*TRAC*	genetically engineering immune cells

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Table 3. *Continued*

Company	Title	Patent no.	Target genes	Diseases/applications
	compositions and methods for epigenetic regulation of *B2M* expression	WO2023250509	*B2M*	genetically engineering immune cells
	compositions and methods for epigenetic regulation of *CIITA* expression	WO2023250512	*CIITA*	genetically engineering immune cells
	compositions and methods for epigenetic editing	WO2023250183	*PCSK9*, *CTLA4*, *VIM3*, *CLTA*	develop epigenetic regulation tools
	compositions and methods for epigenetic editing	WO2022140577	*VIM*	develop epigenetic regulation tools
	compositions and methods for epigenetic regulation of *PCSK9* expression	WO2023215711	*PCSK9*	hypercholesterolemia
	compositions and methods for epigenetic regulation of *ADORA2A* expression	WO2025049789	*ADORA2A*	genetically engineering immune cells
	compositions and methods for multiplex epigenetic regulation	WO2024238689	*B2M*, *CIITA*, *CD247*	develop epigenetic regulation tools
	compositions and methods for epigenetic regulation of *PCSK9* expression	WO2024229020	*PCSK9*	hypercholesterolemia
	compositions and methods for epigenetic regulation of HBV gene expression	WO2024238700	HBV genome	hepatitis B virus (HBV)
	compositions and methods for epigenetic regulation of *CD3gamma*, *CD3delta*, and *RFX5* expression	WO2024238679	*RFX5*, *CD3D*, *CD3G*	genetically engineering immune cells
	compositions and methods for epigenetic regulation of *TGFBR2* expression	WO2025049792	*TGFBR2*	genetically engineering immune cells
	compositions and methods for epigenetic editing	WO2025038840	*FOXP3*, *HELIOS*, *CD25*, *CTLA-4*	develop epigenetic regulation tools
	methods and compositions for multiplex epigenetic editing	WO2025038821	*CD151*, *CTLA-4*	genetically engineering immune cells
	compositions and methods for epigenetic regulation of *RFXAP* expression	WO2025019807	*RFXAP*	genetically engineering immune cells
	methods and compositions comprising DNMT3A-binding antibodies	WO2025030130	*PCSK9*	develop epigenetic regulation tools
Modalis Therapeutics	novel transcription activator	WO2020032057	*MYD88*, *FGF21*, *GCG*	develop epigenetic activation tools
	method for treating facioscapulohumeral muscular dystrophy (FSHD) by targeting *DUX4* gene	WO2022045366	*DUX4*	facioscapulohumeral muscular dystrophy (FSHD)
	method for treating myopathies by targeting Titin gene	WO2023190935	Titin protein coding gene *TTN*	cardiomyopathy
	method for treating Alzheimer's disease by targeting *MAPT* gene	WO2022009987	*MAPT*	Alzheimer's disease
	method for treating muscular dystrophy by targeting *LAMA1* gene	WO2021033635/ WO2022176859	*LAMA1*	muscular dystrophy, particularly merosin-deficient congenital muscular dystrophy (MDC1A)
	method of treating diseases associated with *myd88* pathways using the CRISPR-GNDM system	WO2018212361	*MYD88*	disease associated with activated MYD88 signaling
	method for treating muscular dystrophy by targeting *dmpk* gene	WO2020241903	*DMPK*	muscular dystrophy
	method for treating muscular dystrophy by targeting utrophin gene	WO2020101042	*UTRN*	muscular dystrophy
	method for treating spinocerebellar ataxias (SCA) by targeting *ATXN7* gene	WO2022145495	*ATXN7*	spinocerebellar ataxias (SCA)
	modified Cas9 protein, and use thereof	WO2020085441	*MYD88*	develop epigenetic regulation tools

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Table 3. *Continued*

Company	Title	Patent no.	Target genes	Diseases/applications
	method for treating muscular dystrophy by targeting utrophin gene	WO2021230385	*UTRN*	muscular dystrophy
	method for treating muscular dystrophy by targeting *DMPK* gene	WO2022114243	*DMPK*	muscular dystrophy
	method of treating diseases associated with elevated KRAS expression using the CRISPR-GNDM system	WO2018147343	*KRAS*	cancers with *KRAS* mutation
Epigenic Therapeutics	compositions and methods of genome editing	WO2023165597	*PCSK9*, *CD81*, *CD151*, *VEGFA*	develop epigenetic regulation tools
	method of modulating *PCSK9* and uses thereof	WO2023093862	*PCSK9*	hypercholesterolemia
	method of modulating *VEGF* and uses thereof	WO2023134658	*VEGFA*	*VEGF*-related diseases
	method and use of epigenetic editing target	WO2024032680	*APOC3* gene	*APOC3*-related diseases
	complex and use thereof	WO2024131917	*PTP1B*	*PTP1B*-related diseases
	method for epigenetic editing target and use thereof	WO2024032676	*TTR*	*TTR*-related diseases
	method for epigenetically editing target site and use thereof	WO2024032677	*ANGPTL3*	*ANGPTL3*-related diseases
	fusion and use thereof	WO2024131940	*PCSK9*, *CTLA-4*, *CD151*, *VEGFA*	develop epigenetic regulation tools
	method for epitope editing target and use	WO2024032681	*LPA*	*LPA*-related diseases
	method and use for apparent editing target	WO2024032679	*AGT*	*AGT*-related diseases
	method for epigenome editing of targets and use thereof	WO2024032678	*F11*	*F11*-related diseases
	Cas enzyme and system and use thereof	WO2024251229	*CXCR4*	develop epigenetic regulation tools
	epigenetic editing tool for targeting hepatitis B virus gene	WO2024255823	HBV genome	hepatitis B virus (HBV)
Epicrispr Biotechnologies (Epic Bio)	compositions, systems, and methods for treating familial hypercholesterolemia by targeting *PCSK9*, *LDLR*, and/or *ANGPTL3*	WO2024191731	*PCSK9*, *LDLR*, *ANGPTL3*	hypercholesterolemia
	systems and methods for regulating aberrant gene expressions	WO2024192263	*DUX4*	muscular dystrophy
	systems and methods for regulating aberrant gene expressions	WO2022266324	*DUX4*	muscular dystrophy
	systems and methods for regulating target genes	WO2023004338	*CD71*, *CD45*	develop epigenetic regulation tools
	systems and compositions for fusion polypeptides and methods of use thereof	WO2024092199	*EF1a*, *CXCR4*	develop epigenetic regulation tools
	engineered nucleases, compositions, and methods of use thereof	WO2023168242	*CD2*, *IFNG*, *CXCR4*	develop epigenetic regulation tools
	engineered gene effectors, compositions, and methods of use thereof	WO2023183893	*CD45*, *IFNG*, *CXCR4*, *CD81*	develop epigenetic regulation tools
	systems and methods for genetic modulation to treat ocular diseases	WO2023173120	*RHO*	ocular diseases
	systems and methods for genetic modulation to treat ocular diseases	WO2023172995	*PRPF31*	ocular diseases
	systems and methods for genetic modulation to treat liver diseases	WO2023173072	*SERPINA1*	liver diseases
	compositions, systems, and methods for treating familial hypercholesterolemia by targeting *PCSK9*	WO2023173110	*PCSK9*	hypercholesterolemia

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Table 3. *Continued*

Company	Title	Patent no.	Target genes	Diseases/applications
	systems and methods for engineering characteristics of a cell	WO2022133062	cell characteristics of related genes	engineering cell's characteristics
	systems and methods for regulating target genes	WO2024238661	*GFP*	develop epigenetic regulation tools
Navega Therapeutics	epigenetic gene regulation to treat neurological diseases and pain	WO2022165362	neurological disease and pain related genes	neurological disease and pain
	compositions and methods for epigenetic modulation of NaV1.7	WO2025076459	sodium channel NaV1.7 coding gene	inflammation and pain
	compositions and methods for reducing pain and inflammation through epigenetic modulation	WO2025006508	sodium channel NaV1.7 and NaV1.8 coding genes	inflammation and pain
Omega Therapeutics	compositions and methods for modulating hepatocyte nuclear factor 4-alpha (*HNF4a*) gene expression	WO2021061815	*HNF4a*	liver diseases
	compositions and methods for modulating secreted Frizzled Receptor Protein 1 (*SFRP1*) gene expression	WO2023283359	*SFRP1*	alopecia
	compositions and methods for modulating Forkhead Box P3 (*FOXP3*) gene expression	WO2021183720	*FOXP3*	autoimmune diseases
	methods and compositions for modulating frataxin expression and treating Friedrich's ataxia	WO2021061698	*FXN*	Friedreich's ataxia (FA)
	compositions and methods for modulating apolipoprotein B (*APOB*) gene expression	WO2021061707	*APOB*	hypercholesterolemia
	methods and compositions for modulating *CTNNB1* expression	WO2025019742	*CTNNB1*	cancers
	compositions and methods for reducing *CXCL9*, *CXCL10*, and *CXCL11* gene expression	WO2024243438	*CXCL9*, *CXCL10*, and *CXCL11*	liver disease
	methods for assessing dosage for epigenetic modifying agents	WO2025064469	*MYC*	assessing dosage for epigenetic modifying agents
	methods and compositions for modulating *PCSK9* expression	WO2024238723	*PCSK9*	hypercholesterolemia
	methods and compositions for modulating methylation of a target gene	WO2024238726	not available	regulation of genes expression
Moonwalk Biosciences	controlled reprogramming of a cell	WO2024086673	providing a first and second epigenetic map	provides a method of identifying a target genomic site for epigenetic editing
	methods and compositions for modifying nucleotide sequences	WO2024182440	*CD151*, *CD81*	develop epigenetic regulation tools

The data are dual sourced from the PatentScope and Google Patent database.

applicability and on-target methylation efficiency of epigenome editing tools but lacks a comprehensive interpretation of global off-target activity stemming from the effects of these edits. In engineered mouse embryonic stem cells with double knockout of *Dnmt3a/b* (*de novo* methyltransferase) and transiently repressed *Dnmt1* (maintenance methyltransferase), to deplete DNA methylation, a global hypermethylation induced by dCas9-DNMT3A was evident, with or without sgRNAs.[133] Notably, the catalytic domain of DNMT3A displayed similar global activity, raising concerns that the enforced expression of epigenome editing tools may induce a form of "hypermethylation stress" in the human genome. Although such sequence-independent off-target effects arise primarily from the transient interactions, the additional methyl groups could be preserved and inherited to some extent through cell proliferation, representing the underlying mechanism failed or, more precisely, incomplete to clear such off-target methylation marks.

The varied removal of DNA methylation may relate to local histone modifications or chromosome architecture. Preliminary studies suggest that the persistence of these unintended methylation marks may not be random but could correlate with epigenetic aging processes.[134] Interestingly, reports indicate that incomplete inheritance of on-target DNA methylation marks can occur, with these modifications being lost days after editing,[12,13] although durable and inherited epigenetic changes have also been documented.[11,135,136] Thus, while current literature sheds light on the general applicability and on-target

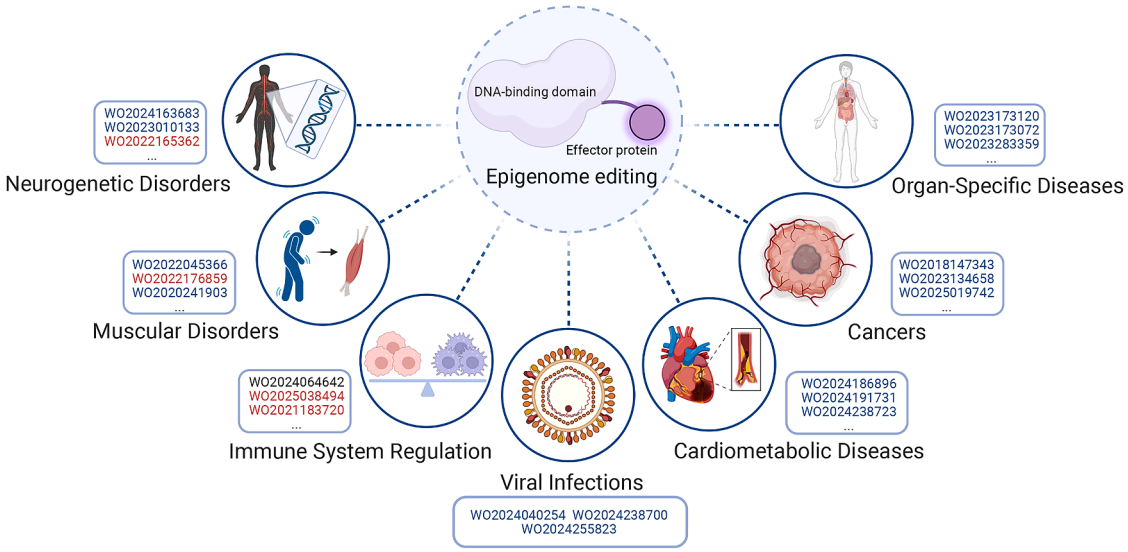

Figure 3. Representative diseases targeted by epigenome editing patents from biotechnology companies

Granted patents from biotechnology companies concentrate on therapies for neurogenetic disorders, muscular disorders, immune system regulation, viral infections, cardiometabolic diseases, cancers, and organ-specific diseases. Representative granted patents shown below respective disease icons. Patent numbers are color coded to indicate the function of the associated epigenome editing tool: red (activation), blue (repression), and black (dual activation/repression). Full details are provided in Table 3.

methylation efficiency, it still lacks a thorough exploration of global off-target activities associated with epigenome editing. Further investigations are warranted to delineate unintended genome interactions from both DNA-binding platform and EE domains.

Maintenance of durable epigenetic edits in proliferation and differentiation

In contrast to genome editing, where genetic alterations are reliably maintained through cell division, induced epigenetic modifications often demonstrate instability over time. The inherent plasticity of the epigenome allows for potential reversibility in gene expression but also renders epigenetic marks relatively transient. Therefore, a critical focus of research is to uncover the mechanisms that can stabilize induced epigenetic changes and ensure their durability.

Due to the interconnected nature of DNA and histone modifications, targeting one of these layers through epigenome editing will inevitably affect the other. Early studies have indicated that targeted DNA methylation induced by dCas9-DNMT3A or dCas9-MQ1 often fails to be efficiently maintained, reverting to baseline levels within days (Figure 4B).[12,13,133] To address this challenge, researchers have explored optimized epigenetic effector variants, such as ETR fusion (DNMT3A, DNMT1, KRAB), combination of DNMT3A3L and KRAB, as well as the CRISPRoff system.[42,135,137] These strategies have shown the potential to sustain epigenetic editing effects for weeks to months. Notably, the KRAB domain initiates a cascade of repressive histone modifications and chromatin remodeling, such as the removal of histone H3K4me3 and acetyl groups, creating a stable repressive environment that supports the maintenance of induced DNA methylation patterns. Consequently, the synergistic editing of both DNA methylation and histone modifications, utilizing a combination of effector domains (e.g., dCas9-DNMT3A/DNMT1 and dCas9-KRAB), can significantly enhance the silencing effect and bolster the long-term repression of target gene expression.[138] Recent breakthroughs include the successful repression of endogenous *Pcsk9* gene expression for months.[46,53] Further research indicated that, when co-expressed with DNMT3A and DNMT3L, Ezh2-dCas9 outperformed KRAB-dCas9 in enabling long-term silencing,[16] while the targeted acetylation alone is more than sufficient for gene activation.[18,139] This emphasizes the necessity for a systematic exploration of optimal combinations of DNA methylation and histone modifications to attain desired epigenome editing outcomes.

Although multi-component systems have demonstrated remarkable long-term effects, their complexity may pose challenges for *in vivo* therapeutic applications. Ongoing research is also investigating the potential of DNA methylation editing as an independent approach. One study demonstrated that DNA methylation editing via dCas9-DNMT3A3L is heritable in primary human hematopoietic stem and progenitor cells and persists through hematopoiesis *in vivo*.[140] The engraftment model showed that the edited epigenetic marks were conserved and inherited across both myeloid and lymphoid lineages, suggesting fidelity during proliferation and differentiation. Recent investigations employing the CHARM system to recruit and activate endogenous, auto-inhibited DNMT3A protein achieved long-lasting gene silencing in the brain, indicating that DNA methylation alone may effectively suppress gene expression without KRAB support.[57] Therefore, understanding the intricate interplay between DNA and histone modifications, along with the

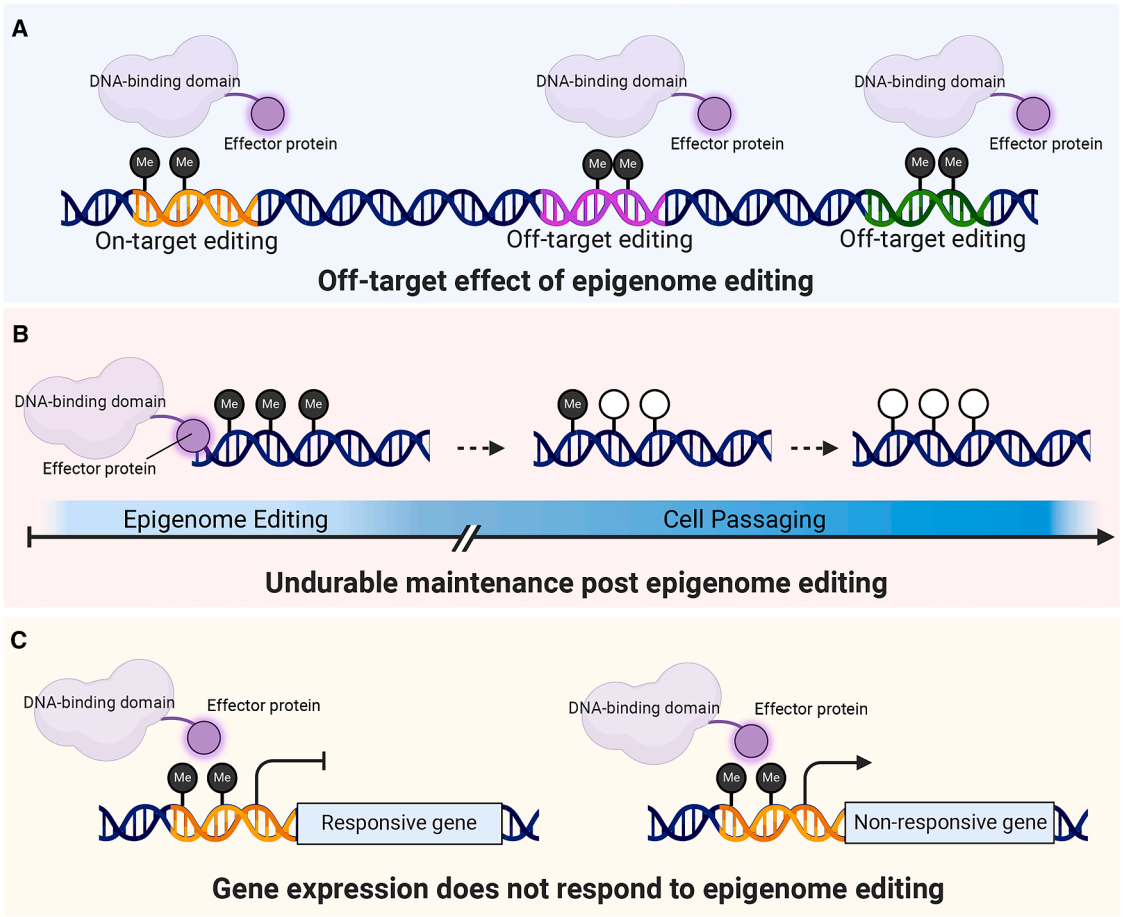

Figure 4. The challenges of epigenome editing

Key challenges in epigenome editing include: (A) potential off-target effects, (B) undurable epigenetic modifications, with progressive loss during cellular proliferation, and (C) failure to establish functional gene regulation despite successful on-target epigenetic modifications.

careful optimization of epigenetic effectors, remains crucial in this rapidly advancing field.

Epi-editing insufficiency in altering gene expression

Genome editing-mediated disruptions usually lead to clear biological outcomes. For example, the indels at TF binding sites can directly alter gene expression by inhibiting regulatory factor binding. Similarly, frameshift mutations in coding regions lead to truncated or unstable protein products. However, the effects of epigenome editing are often more nuanced and context dependent. Induced DNA methylation does not always yield the anticipated changes to target gene expression, partially due to limited methylation coverage of the promoter region (Figure 4C).

Advanced epigenome editing tools, such as dCas9-DNMT3A3L or CRISPRoff, can enhance gene silencing by broadening the range of DNA methylation and ensuring its durability. However, even with significant hypermethylation, the correlation between methylation levels and target gene repression is not always straightforward.[10] Potential explanations include distinct spatial bindings of TF complexes at promoters or enhancers, as well as variable durability of the silencing effects across different target genes. The *PCSK9* gene, for example, was efficiently silenced for over 100 cell passages using CRISPRoff, while the silencing effect was less durable using the same tool for other genes such as *ANGPTL3* and *AGT*.[44] Consequently, the causal relationship between epigenetic modifications and long-lasting gene expression changes is context dependent, and it may not be generalizable across all target genes. Achieving a more durable and efficient gene silencing strategy remains a case-by case attempt, particularly regarding the need for induced hypermethylation to cover a significant proportion of the promoter region (e.g., over 80% CpGs) or to fully methylate critical CpG sites.

An alternative strategy for manipulating gene expression through DNA methylation editing is based on the idea that certain DNA elements or TF binding sites are sensitive to methylation. These TF binding sites are typically narrow with a relatively clear background, enabling long-range regulatory effects spanning thousands of base

pairs. Early studies showed that hypermethylation at methylation-sensitive CTCF binding sites failed to obtain detectable biological effects *in vivo*.[13] To address this limitation, a combination of dCas9-KRAB and dCas9-DNMT3A3L was employed to effectively disrupt CTCF binding and alter the topological mechanisms of gene regulation. This approach has been further applied *in vivo* using the SunTag-TET1 catalytic domain to generate mouse models of the Silver-Russell syndrome (SRS).[141] The introduction of gRNA for *Igf2/H19* imprinting control region was sufficient to reactivate *H19* and reduce *Igf2* expression to mimic the expression dysregulations in SRS patients, suggesting that the regulatory element but not the promoter may be essential for governing expression through epi-editing.

Alternative approaches to induce methylation changes

Within the cell nucleus, DNA methylation is tightly regulated by endogenous machinery, primarily the DNMT and TET protein families. Understanding how to harness this endogenous system represents an intriguing area of research. Studies have highlighted that DNA methylation is integral to the gene silencing process during viral latency,[142,143] indicating that inserting specific DNA fragments could potentially trigger endogenous hypermethylation. Takahashi et al. developed a technique to introduce a CpG-free DNA fragment into a CpG island in human stem cells, leading to induced methylation of the entire targeted region.[144] Notably, the *Cre-loxP* mediated removal of the CpG-free cassette did not affect the acquired methylation or the expression of the targeted gene, even after more than 20 passages. Building on this stem cell tool, the authors recently expanded their work to investigate transgenerational epigenetic inheritance in mice. By targeting CpG islands in promoters using CpG-free fragments in mouse embryonic stem cells and injecting the edited cells into eight-cell stage embryos, they demonstrated that induced DNA methylation was stably maintained during embryonic development and transmitted from parents to offspring across at least four generations. The targeted *Ankrd26* gene expression was silenced, leading to metabolic dysregulation, including obesity and hypercholesterolemia.[145] Although a persistent genomic change (a TTAA tetra-nucleotide remains) was integrated into the host genome and the underlying mechanism for these abrupt epigenetic change remains to be elucidated, this "minimal scar" approach offers an alternative to traditional fusion protein-based methods for targeted DNA methylation.

Another promising approach to programmable control gene expression is the CRISPR-genome organization (CRISPR-GO) system (Figure 1H), which is a chemically inducible and reversible, enabling interrogation of real-time dynamics of chromatin interactions with nuclear compartments.[146,147] This system utilizes ligand-mediated dimerization of two domains: one coupled with the CRISPR-dCas9 system to bind target loci and the other fused with nuclear compartment-specific proteins. By using the ligand as a switch, the targeted genome can be physically relocated to the inner surface of the nucleus, specifically into lamina-associated domains characterized by gene repression and abundant H3K9me2/3 heterochromatin marks. After repositioning the targeted genome to the nuclear periphery, the CRISPR-GO system successfully induced a consistent decrease in reporter and endogenous gene expression compared with untreated cells. The detailed molecular mechanisms underlying this gene repression, including potential changes in epigenetic marks such as DNA methylation or histone modifications, and the durability of these epigenetic changes, remain to be investigated. If gene silencing could be traced by epigenetic marks, the causal relationships between 3D genome organization and human diseases may inspire real-time gene therapy approaches using the CRISPR-GO system.

Appropriate scenario for epigenome editing

A fundamental issue in epigenome editing revolves around the unique advantages it offers compared with traditional genome editing, as well as the most appropriate scenarios for its application. One key advantage is the ability to restore altered expression of targeted genes in human diseases to optimal levels. Additionally, the reversibility of epigenetic modifications, such as DNA methylation and histone marks, enables fine-tuning and modulation of gene expression patterns in response to various cellular and environmental signals, rather than imposing fixed, permanent changes. Potential therapeutic targets for epigenome editing include genetic and chronic diseases, such as imprinting disorders (e.g., Beckwith-Wiedemann syndrome, and SRS), fragile X syndrome, neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease), and certain viral infections. However, many of these applications remain in the proof-of-concept stage and are far from mature clinical implementation. The advantages of using epigenome editing are marginal compared with genome editing in several aspects at current stage. Furthermore, long-term efficacy and stability can raise apprehensions regarding the need for repeated treatments. Methylation heterogeneity after editing in stem cells may influence cell fate determination and complicate functional evaluation.

OUTLOOK

The challenges commonly faced in genome editing, including efficient and tissue-specific delivery, post-therapy self-silencing or removal, and minimized off-target effects, also apply to the field of epigenome editing. The combined utility of genome and epigenome editing is garnering increasing attention. For example, a recent study suggested that epigenome editing could alter the efficiency of prime editing at the targeted loci.[148] Subsequently, we outline key considerations shaping the field's future.

Promising therapeutic applications

Epigenome editing holds transformative potential in following key areas: (1) silencing multi-copy pathogenic elements (e.g., HBV cccDNA, MYC amplifications, CAG repeat in Huntington's disease), without inducing DNA double-strand breaks. This contrasts with CRISPR-Cas9-mediated cleavage, which risks chromosomal rearrangement and genome instability.[149–151] (2) Reversibly alleviating chronic pain to avoid permanent sensory loss.[111] Genome editing, causing permanent pain insensitivity, may raise safety and ethical concerns in clinical applications. (3) Rescuing haploinsufficiency disorders (e.g., Titin truncating variants [TTNtv] cardiomyopathy)

through allele-specific activation.[152] (4) Correcting aberrant epigenetic silencing in cancer (*CDKN2A/MLH1* reactivation),[153] imprinting disorders, and neurodevelopmental diseases (*MECP2* demethylation in Rett syndrome).[154] By enabling targeted epigenetic activation or demethylation, these tools can restore functional gene dosage in haploinsufficiency disorders and reactivate genes silenced by pathological epigenetic mechanisms.

Essential technological advancements for clinical adoption

Clinical adoption requires several key technological advancements, including tissue-specific delivery systems (e.g., AAV and LNP),[155–157] tool miniaturization, and self-silencing post-treatment. Effective delivery platforms need to achieve high tissue specificity and safety through advanced spatiotemporal control technologies, such as optogenetic switches or small-molecule-inducible systems.[147,158] Tool miniaturization is also crucial. Current bulky dCas9 fusion proteins, which exceed 4.2 kDa, pose challenges for *in vivo* delivery. Emerging solutions include TIGR-Tas,[159] Fanzor,[160] and computational modeling-based rational design,[52] which hold promising frameworks to optimize genome-wide epigenetic perturbation.[99] Chronic transgene expression from AAV-delivered constructs in non-dividing cells raises concerns about undesirable durable expression and off-target concerns. Integrated self-silencing mechanisms (e.g., CHARM) are essential to terminate editor activity post-treatment, enhancing therapeutic safety.[57]

Regulatory and ethical considerations

Although epigenome editors do not directly cleave DNA, their off-target effects may induce genome-wide epigenomic perturbations, including aberrant methylation spreading and histone modification diffusion. Consequently, rigorous long-term safety assessments are essential. These should incorporate longitudinal follow-up over years, combined with single-cell multi-omics technologies to dynamically monitor epigenetic drift. Regulatory authorities must establish independent frameworks specific to epigenome editing, encompassing genome-wide methylation profiling, 3D chromatin architecture analysis, and cell fate potential assessments. Ethically, germline epigenome editing raises unique concerns due to the potential for transgenerational epigenetic inheritance (e.g., through mitochondrial mechanisms), which could exacerbate "designer baby" controversies. Additional attention is warranted for edits affecting cognition-related genes (e.g., *BDNF* promoter methylation levels), which modulate learning capacity.[161,162] Non-therapeutic applications should therefore be strictly restricted.

The future of epigenome editing in clinical treatment holds great promise, fueled by foundational research and innovative proof-of-concept studies. Significant progresses in delivery technologies and long-term effect *in vivo* have brought us closer to realizing safe and effective epigenome therapies, although broader applications remain elusive. With numerous refinements in tool development and validation studies, this innovative approach is on the edge of entering clinical practice, promising to establish a novel therapeutic avenue for safely addressing diseases with limited treatment options.

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AUTHOR CONTRIBUTIONS

Y.L. conceived the topic. L.W.Y., Y.K.X., and Y.M.Z. collected literatures. L.W.Y. and Y. L. took the lead in writing the manuscript. All authors contributed edits and provided critical feedback that helped shape the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT in order to polish the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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