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Epigenome Editing: Emerging Tools, Therapeutic Applications, and Challenges in Human Disease Treatment

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Abstract

Epigenetic modifications, including histone alterations, non-coding RNA interactions, and DNA methylation, regulate gene expression without altering the underlying DNA sequence. These modifications are essential for normal biological processes; however, their aberrant regulation is linked to numerous life-threatening disorders. Genome editing nucleases such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas systems offer promising tools for the precise correction of epigenetic abnormalities. This review explores epigenetic mechanisms, genome editing technologies for epigenetic modulation, and their applications in disease contexts, such as cancer and neurodegeneration, with reference to both *in vitro* and *in vivo* studies demonstrating therapeutic potential. For instance, aberrant histone acetylation and methylation patterns are frequently observed in cancer. Abnormal DNA methylation and disruptions in histone modifications have been implicated in neurological disorders, such as Alzheimer's and Huntington's disease. Although ZFNs and TALENs are foundational tools, their use has been limited by challenges in protein engineering and nonspecific targeting. CRISPR/Cas systems have become a versatile platform. Catalytically inactive Cas9 (dCas9) can be fused to epigenetic editing domains, such as histone deacetylases and DNA methyltransferases, to precisely regulate gene expression. For example, dCas9 has been used to reactivate the *BRCA1* tumor suppressor gene in cancer cells. Although epigenetic editing holds significant promise in biomedical research and precision medicine, several challenges remain. These include unintended epigenetic alterations, the efficient delivery of editing tools to target cells, and limited *in vivo* validation. Future studies using animal models are essential to evaluate the translational potential and clinical applicability of this approach.

Introduction

Epigenetics refers to heritable alterations in the chromatin structure that influence gene expression without modifying the underlying DNA sequence. These modifications are primarily mediated by DNA

methylation and histone modifications, which together regulate transcriptional activity in a context-dependent manner (Wu et al., 2023b). DNA methylation typically occurs in CpG dinucleotide islands and is commonly associated with transcriptional repression (Liesenfelder et al., 2025). However, it can also enhance transcription in specific genomic regions such as introns by recruiting histone modifiers and chromatin remodelers (Dhar et al., 2021). This dual role demonstrates the value of DNA methylation as a dynamic regulatory mechanism for eukaryotic gene expression.

Wu et al. (2023a) demonstrated that, in eukaryotic cells, DNA is organized into a dynamic chromatin structure through the formation of nucleosomes, each comprising a histone octamer (H2A, H2B, H3, and H4) wrapped by 146 base pairs of DNA. Histone proteins undergo various post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation, which influence chromatin accessibility and transcriptional regulation.

Aberrant epigenetic modifications have been implicated in numerous diseases, including cardiovascular diseases, neurological disorders, and cancer (Robusti et al., 2022). For example, global hypomethylation and gene-specific hypermethylation are common in cancer, whereas atypical histone acetylation patterns have been observed in neurodegenerative diseases. These epigenetic abnormalities make these disorders promising targets for therapeutic intervention via epigenome editing. Given the reversible nature of epigenetic marks and their central role in cellular function, the targeted modulation of gene expression has emerged as a compelling strategy for gene therapy and cellular reprogramming.

To manipulate these modifications for therapeutic purposes, genome-editing tools have emerged as powerful platforms for targeted epigenetic regulation. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats and associated proteins (CRISPR/Cas9) have significantly advanced the precision and versatility of epigenetic modulation (Dehshahri et al., 2021; Ueda et al., 2023). ZFNs and TALENs recognize target sequences via engineered protein domains, making their design labor-intensive and costly (Bayat et al., 2017). In contrast, CRISPR/Cas9 is guided by a customizable 20-nucleotide RNA sequence that enables rapid and scalable targeting (Bayat et al., 2024a; Bayat et al., 2024b; Shams et al., 2022).

The fusion of epigenetic modifiers with genomic editing platforms has led to epigenome editing. A diverse array of epigenome editing effectors has been conjugated into genome-editing tools and is generally categorized into two primary groups: enzymatic effectors, such as p300, and non-enzymatic effectors, such as VP1 (Zhang et al., 2025).

Types of epigenetic modifications

Epigenetic modifications can be categorized into three primary classes: (i) histone modifications, (ii) DNA methylation, and (iii) non-coding RNA (ncRNA)-mediated mechanisms (Figure 1).

Histone modifications

The histone code consists of a diverse array of post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation. Histone acetyltransferases (HATs) catalyze the addition of acetyl groups to lysine residues on histones H3 and H4, which typically enhance gene expression. In contrast, histone deacetylation represses transcription. Key cellular components with HAT activity include p300/CBP (CREB-binding protein) (Kikuchi et al., 2023), SAGA complex (Spt-Ada-Gcn5 acetyltransferase) (Meriesh et al., 2020), and TAF1 (TATA-Box Binding Protein Associated Factor 1) (Kloet et al., 2012). The methylation of arginine and lysine residues in histones H3 and H4 is facilitated by histone methyltransferases (HMTs). Domains such as G9A, SUV39H1, KRAB, DNMT3A, Ezh2, and Friend of GATA-1 (FOG1) are commonly used to modulate histone methylation patterns (O'Geen et al., 2017). Phosphorylation occurs on threonine, serine, and tyrosine residues, particularly within histone H3, which plays a central role in the chromatin structure. Phosphorylation introduces negatively charged phosphate groups that disrupt histone-DNA interactions, thereby facilitating transcription (Liu et al., 2023).

Histone ubiquitination involves the attachment of ubiquitin to histones H2A and H2B via histone ubiquitin transferases. The ubiquitination of H2B is linked to transcriptional activation, whereas H2A ubiquitination is associated with transcriptional repression (Morgan & Wolberger, 2017). SUMOylation, the conjugation of small ubiquitin-like modifiers (SUMO) to lysine residues, contributes to transcriptional repression and chromatin compaction. SUMOylation has been observed in histones H2A, H2B, H3, H4, and H1 (Ryu & Hochstrasser, 2021; Ryu et al., 2020). Poly (ADP-ribose) polymerase (PARP) catalyzes poly (ADP-ribosyl)ation (PARylation). The mono-ADP-ribosylation of core histones and histone H1 has been documented, and this modification promotes transcription by facilitating chromatin remodeling (Martinez-Zamudio & Ha, 2012).

DNA methylation

DNA methylation, facilitated by DNA methyltransferases (DNMTs), is a key epigenetic mechanism that is commonly associated with transcriptional repression (Loscalzo & Handy, 2014). In eukaryotes, 5-methylcytosine (5mC) is the predominant methylation marker (Li, 2021). The ten-eleven translocation (TET) enzyme family reverses cytosine methylation by converting 5mC to

hydroxymethylcytosine, followed by further oxidation into 5-formylcytosine and 5-carboxylcytosine. These oxidized bases are subsequently removed via DNA glycosylation and the base-excision repair pathway (Castro-Munoz et al., 2023).

ncRNA related mechanisms

Non-coding RNAs (ncRNAs) also play pivotal roles in regulating epigenetic processes. *XIST*, a 17-kb long ncRNA, coats the X chromosome designated for inactivation and initiates gene silencing. A shorter transcript from the *Xist* locus, *Rep A*, is functionally critical for recruiting polycomb repressive complex 2 (PRC2), which catalyzes histone H3 lysine 27 trimethylation (H3K27me3), a hallmark of transcriptionally silent chromatin (Loda & Heard, 2019). Nuclear long ncRNAs (lncRNAs) further modulate chromatin architecture by guiding chromatin-modifying complexes to specific genomic loci (Morlando & Fatica, 2018). MicroRNAs (miRNAs) regulate gene expression post-transcriptionally by binding to the 3' untranslated regions (3'UTRs) of target mRNAs, thereby influencing mRNA stability and translation. They also indirectly affect epigenetic states by modulating the expression of enzymes such as histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) (Ramzan et al., 2021).

While DNA methylation and ncRNAs are distinct regulatory layers, their interplay with histone modifications, as discussed in the preceding section, suggests a coordinated epigenetic network that governs gene expression and cellular identity. Table 1 summarizes the major epigenetic modifications, their enzymatic mediators, and their associated disease contexts.

Epigenetic editing

Genome editing tools are reprogrammable enzymes that target specific DNA sequences (Figure 2). A summary of the epigenetic editing tools is presented in Table 2.

ZFNs

Homologous recombination has traditionally served as the primary method for targeted integration of genes of interest in host genomes. The discovery of reprogrammable endonucleases with DNA-binding capabilities has significantly transformed genome engineering (Bayat et al., 2018). Among novel gene editing technologies, ZFNs were the first to be developed through protein engineering, possessing the ability to edit specific genomic regions (Laufer & Singh, 2015; Li et al., 2020a). ZFNs are composed of tandem repeating protein modules with an α-helical structure that bind to the target DNA by recognizing the major groove. These modules confer specificity to the target site within the genome, with each module recognizing 3–4 base pairs (bp). Typically, ZFNs target sequences range from 9 to 18 bp depending on the number of zinc finger modules used. Specifically engineered zinc fingers are fused to FokI, a type IIs restriction endonuclease naturally occurring in *Flavobacterium okeanokoites* that cleaves DNA upon dimerization (Chandrasegaran, 2017; Urnov et al., 2010). Typically, multiple zinc-finger modules are designed on either side of the target site. Snowden et al. (2002) were the first to use ZFN technology to edit epigenetic codes, specifically H3K9 methylation. They employed an engineered ZFN, in which a catalytically inactive ZFN was fused to an H3K9 histone methyltransferase, to examine its impact on *VEGFA* expression. These results demonstrated that H3K9 methylation exerts a repressive effect on target genes. However, the engineering of zinc-finger arrays presents significant challenges. To address this issue, Ichikawa et al. (2023) screened 49 billion protein-DNA interactions and developed a deep-learning model called ZFDesign to engineer specific ZFNs.

TALENs

TALENs were initially identified in the plant pathogenic bacterium Xanthomonas. Their DNA-binding domains are composed of 33–35 highly similar repeat units, with each repeat unit recognizing a single base pair. This characteristic enhances the specificity of TALENs compared with that of ZFNs. The specificity of TALENs is determined by the amino acid composition of the repeat variable di-residues (RVD) located at positions 12 and 13 of each repeat (Sanjana et al., 2012). Owing to the simplicity of the recognition code and design flexibility, TALEN-based gene editing is more feasible than ZFNs (Gaj et al., 2013; Khan, 2019). Similar to ZFNs, TALENs have been engineered to manipulate the activation and repression of target genes. Maeder et al. (2013a) fused the TET1 demethylase effector to an enzymatically inactive TALEN to investigate the effects of methylated promoters at CpG positions on the expression of downstream genes. This engineered TALEN system specifically targeted 20-bp sites in the hemoglobin subunit beta (*HBB*) gene, effectively demethylated CpG islands, and induced beta-globin expression. Despite the efficiency of ZFNs and TALENs platforms for epigenetic editing, the extensive efforts required for protein engineering, associated costs, and high off-target effects have limited their use (Gaj et al., 2013; Maeder et al., 2013b).

CRISPR technology

CRISPR systems were originally discovered as adaptive immune mechanisms in bacteria and archaea, where they facilitate the recognition and degradation of invading genetic elements, such as phages and plasmids. These systems can be classified into two primary classes and types. Class II type II CRISPR systems, particularly CRISPR/Cas9, have been optimized for genome and epigenome editing in mammalian cells. This system comprises an effector protein, Cas9, and guide RNA, which includes CRISPR RNA (crRNA) (20 nt) and trans-activating CRISPR RNA (tracrRNA) (Salmaninejad et al.,

2018). The CRISPR/Cas9 system has been refined for application in mammalian cells to target regions containing canonical NGG protospacer motif sites (Bayat et al., 2018; Salmaninejad et al., 2021). Analogous to ZFNs and TALENs, CRISPR/Cas9 technology has been employed to modulate transcriptional activation, repression, and epigenetic modifications at specific target sites. Typically, epigenetic modifications are executed using a catalytically inactive Cas9 endonuclease referred to as dCas9 (Nakamura et al., 2021). When epigenetic editing domains such as DNMT and HDAC are fused to dCas9 and associated with a specific guide RNA, they can precisely regulate the expression and repression of target genes (Gjaltema & Rots, 2020). In a previous study, dCas9 was fused to the histone demethylase LSD1 to investigate new functional enhancers in the embryonic stem cell state, particularly those that regulate *OCT4* expression (Kearns et al., 2015). CRISPR-based tools can overcome these limitations and facilitate precise and rapid assessment of cis-regulatory elements by directing specific epigenetic editing domains to target sites.

Compared to ZFNs and TALENs, CRISPR/dCas9 systems are now favored because of their ease of design, higher specificity, scalability, and multiplex ability, allowing simultaneous targeting of multiple genomic loci.

A comparative assessment of epigenome editors

Extensive research on synthetic zinc finger (ZF) proteins has underscored the advantages of these DNA-binding domains. Their compact structure facilitates efficient delivery, ensures elevated expression levels, and enables epigenetic modifications across diverse chromatin environments, including regions characterized by heavily methylated DNA (Katayama et al., 2024). Intrathecal administration of a ZF-KRAB repressor via adeno-associated virus (AAV) in non-human primates resulted in up to 60% repression of *Scn9a* expression. This treatment was well tolerated in non-human primates, with no dose-limiting adverse effects observed four weeks after a single intrathecal injection (Samie et al., 2024). In contrast, a zinc finger artificial transcription factor targeting *VEGF* for the treatment of diabetic neuropathy, based on transcriptional activation rather than epigenome editing, progressed to phase II clinical trials but failed to demonstrate a therapeutic effect compared to placebo (Eisenstein, 2012). A significant concern, especially for ZF-based epigenetic editing tools that might be considered for clinical trials, is the risk of off-target effects due to their propensity for indiscriminate binding. High-throughput profiling has revealed that ZFs can bind thousands of unintended genomic sites, with off-target frequencies ranging from 10% to 40%, depending on the construct and cell type (Seem et al., 2024). Furthermore, the incorporation of effector domains can alter ZF binding patterns; for example, adding a KRAB domain to ZF has been demonstrated to increase off-target binding, particularly in regions outside promoters (Seem et al., 2024). Research on ZF-based editors has predominantly employed ectopic overexpression, which may result in the recognition of unintended genomic sites. In natural systems, it is likely that ZF protein expression is regulated both spatially and temporally within complex transcriptional networks, thereby enabling the precise modulation of gene expression and phenotype determination (Zhou et al., 2025). Nevertheless, insights derived from these pioneering DNA-binding domains have facilitated more effective utilization of subsequent epigenome editing platforms, which may offer improved DNA-recognition specificity.

Unlike ZFs, transcription activator-like effector (TALE)-based epigenome editing tools demonstrate minimal off-target cleavage. Mendenhall et al. (2013) introduced a strategy employing a fusion editor, TALE-LSD1, to facilitate the demethylation of histones at endogenous regulatory elements within the stem cell leukemia locus, which is enriched for histone marks such as H3K4me2 and H3K27ac in K562 erythroleukemia cells without detectable off-target effects. Nonetheless, multiple studies have reported low yet detectable levels of off-target cleavage, both *in vitro* and *in vivo* (Becker & Boch, 2021). Research has demonstrated that the binding efficacy of TALEs is markedly reduced in the presence of hypermethylated DNA. For example, TALE-VP16 fusions targeting *Oct4* were successful in binding and augmenting gene expression in embryonic stem cells; however, they were ineffective in ESC-derived neural stem cells owing to hypermethylation at the target promoter (Hu et al., 2014). Nevertheless, researchers have identified novel RVDs capable of recognizing and binding to methylated DNA, including those with the amino acid codes NG, N*, HA, or R* (Zhang et al., 2017). These RVDs can be incorporated into TALE or TALEN constructs to enable genome editing, which is contingent on methylation (Becker & Boch, 2021). The modular structure of TALEs makes their design easier for target sites, and their large-scale and quick assembly has made them the preferred option for high-throughput studies compared to ZFs. However, because of the presence of numerous tandem repeats in TALEs, their cloning and delivery, especially using lentiviral plasmids (increased susceptibility to deletions and recombination), have encountered serious challenges (Mock et al., 2014). Recent advances in delivery platforms, such as mRNA-based systems and nanoparticle carriers, may help mitigate these barriers and improve the TALE delivery efficiency.

In contrast to TALEs, the primary benefit of CRISPR technology is the ease with which new single guide RNAs (sgRNAs) may be generated, rather than the significant time and skill requirements involved in developing new protein-based DNA-binding domains. Compared to ZFs and TALEs, this characteristic offers a significant targeting variety and is probably a key factor in the rapid

development of the CRISPR technology. This technology has also been demonstrated to have off-target effects in various studies. It has been indicated that the Cas9 protein can tolerate up to five mismatches at the sgRNA binding site (Bayat et al., 2017). The reported off-target frequencies of CRISPR-based systems vary significantly. Even when a significant number of off-target binding sites are found, epigenome editing techniques using dCas9 fusions are typically restricted to the on-target site in a specific manner, thus minimizing unintended chromatin remodeling (Cappelluti et al., 2024; Tremblay et al., 2025). Alternative Cas proteins, which may exhibit superior editing selectivity compared to the conventional SpCas9, could be employed to circumvent this limitation. For example, the Cas protein Cpf1 recognizes a 5′ TTN PAM and utilizes a shorter crRNA (Bayat et al., 2018). A notable advantage of employing Cpf1 is its requirement for only a short crRNA, as opposed to a crRNA-tracrRNA complex, and its ability to process its precursor crRNA through RNase activity. This capability facilitates delivery of multiple crRNAs to cells in a single array (van Esch et al., 2025). Additionally, high-fidelity Cas9 variations that produce Cas9 proteins with no discernible off-target effects are produced by mutations in residues that typically create non-specific interactions with DNA (Bayat et al., 2024b; Skeens et al., 2024; Tang et al., 2022). When combined with epigenome editor domains, such as KRAB or TET1, these high-fidelity variants unlock new potential for precise and programmable chromatin remodeling.

Applications for epigenetic editing

Epigenome editing studies serve multiple purposes in both basic research and therapeutic development. The primary objectives of basic research are to elucidate the activity of effectors at specific genomic loci and identify the locations and consequences of epigenetic modifications. For instance, the fusion of SMYD3, a lysine methyltransferase, with dCas9 has clarified the role of this enzyme in the methylation of H3K4 and H4K5 (Kim et al., 2015). Such studies will enhance our understanding of chromatin dynamics and gene regulation in both physiological and pathological contexts.

The implementation of inducible promoters offers a robust strategy for temporally controlling CRISPR-based epigenetic editing tools and assessing their functions in dynamic systems (Li et al., 2020c). Common inducible systems include doxycycline-responsive promoters and light-inducible dCas9 constructs, which allow the precise modulation of effector activity *in vitro* and *in vivo* (Altinbay et al., 2024; Zhang et al., 2019).

Table 3 presents a compilation of the CRISPR/Cas9-based epigenetic editing tools. An overview of the key studies employing these tools to develop therapeutic strategies for human diseases is provided below.

Cancer

Recent studies have demonstrated that epigenetic dysfunction plays a significant role in the development of malignancies. It is plausible that DNA hypomethylation in the promoters of oncogenes or hypermethylation in the promoters of tumor suppressor genes contributes to cancer progression (Castro-Munoz et al., 2023; Costa et al., 2023; Lu et al., 2020). In cancer cells, aberrant expression of chromatin-modifying enzymes is common, and HDACs are often overexpressed, while HATs are downregulated (Gu et al., 2024).

However, histone methylation also has context-dependent effects. For example, H3K27me3 is associated with transcriptional repression and is frequently elevated in aggressive cancers such as glioblastoma and prostate cancer. In contrast, H3K4me3 correlates with active transcription and is often dysregulated in leukemia and breast cancer (Chen et al., 2020). These markers serve as critical indicators of chromatin state and therapeutic targets.

Saunderson et al. (2023) developed a CRISPR-based DNA methylator, dCas9-3A3L, to modify the promoters of *CDKN2A* and *CDKN2B* in human stem/progenitor cells. Their findings revealed that the induced epigenetic changes were heritable, suggesting their utility in disease modeling and regenerative medicine. Similarly, the dCas9-TET1 demethylase tool reactivates the *BRCA1* tumor suppressor gene in cervical and breast cancer cells, leading to reduced tumorigenesis (Choudhury et al., 2016).

Although cancer remains a primary focus, epigenetic editing tools are increasingly being explored in other disease contexts. For instance, aberrant DNA methylation and histone modifications are implicated in neurological disorders, such as Rett syndrome and Alzheimer's disease, imprinting disorders, such as Prader-Willi syndrome, and immune conditions, including lupus and multiple sclerosis. These applications highlight the versatility of CRISPR-based epigenetic editing tools for modulating gene expression across diverse biological systems.

Neurological disorders

Neurological diseases represent a diverse group of disorders prevalent in the population. The etiology of these disorders is multifactorial and involves genetic and epigenetic alterations, environmental influences, physical injury, and disease-associated inflammation (Migliore & Coppede, 2009; Mirahmadi et al., 2025). Recently, the contribution of epigenetics to neurological diseases has been a subject of extensive research. Mutations in epigenetic regulators, such as MeCP2, in Rett syndrome can directly cause disease, whereas in other cases, epigenetic marks are dysregulated as a consequence of pathological processes (Singh & Santosh, 2025). Mutations or alterations in proteins that regulate epigenetic mechanisms are linked to various neurological disorders, including autism, Alzheimer's

disease, Huntington's disease, Rett syndrome, Rubinstein-Taybi syndrome, ATRX syndrome, and Friedreich's ataxia, among others. Aberrant DNA methylation patterns, disruptions in histone modifications, and changes in chromatin remodeling factors such as DNMTs, MBDs, HDACs, HATs, HMTs, HDMs, and the SWI/SNF family are critical proteins implicated in the onset and progression of neurological diseases (Berdasco & Esteller, 2013; Jakovcevski & Akbarian, 2012). While most studies have not identified specific epigenetic modifications associated with Alzheimer's disease, epigenome-wide methylation analyses have revealed significant variations in DNA methylation across different brain regions in Alzheimer's disease, utilizing human post-mortem samples (Lunnon et al., 2014). Furthermore, research involving monozygotic and dizygotic twins has demonstrated a correlation between epigenetic modification of the *ADARB2* gene and the pathogenesis of Alzheimer's disease (Sharma et al., 2020).

Adult anxiety is often modulated by the synaptic activity response element (*SARE*) located near the activity-regulated cytoskeleton-associated protein (*ARC*) gene in adolescents exposed to alcohol (Bohnsack et al., 2022). To elucidate this relationship, the effects of dCas9-p300 (a histone acetylation activator) and dCas9-KRAB (a transcriptional repressor) were investigated in animal studies. Alterations in histone acetylation or methylation at *Sare* result in increased or decreased expression of *Arc* genes, thereby influencing anxiety in a rat model of adolescent alcohol exposure (Blum et al., 2024; Bohnsack et al., 2022). This finding underscores the utility of CRISPR-based epigenetic editors as tools for exploring mechanisms underlying complex neurological disorders. In a separate study, the CRISPR activator system was effectively employed for multiplex activation of three neural growth factors (*NGF*, *BDNF*, and *GDNF*) in adipose stem cells, which enhanced peripheral nerve regeneration in a rat model of sciatic nerve injury (Hsu et al., 2019).

Collectively, these studies highlight the pivotal role of diverse epigenetic modifications in the pathogenesis of neurological disorders, ranging from neurodevelopmental to neurodegenerative conditions. The emerging use of CRISPR-based epigenetic editing tools, as evidenced in research on anxiety and nerve regeneration, provides powerful tools not only for elucidating the complex epigenetic mechanisms underlying these conditions but also for developing targeted interventions aimed at restoring neurological functions. To provide a balanced perspective, we briefly noted challenges such as epigenetic heterogeneity across brain regions and the blood-brain barrier as delivery limitations. Additionally, we acknowledge ethical considerations and the potential for long-term transcriptional reprogramming when modulating genes in the central nervous system (CNS).

Autoimmune diseases

Autoimmune diseases, including multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM), ankylosing spondylitis (AS), and inflammatory bowel disease (IBD), arise from the inability of the immune system to tolerate self-antigens. Epigenetic mutations significantly influence immune system components such as T cells, antibodies, major histocompatibility complexes (MHCs), and cytokines, thereby playing a crucial role in the onset and progression of autoimmune diseases (Richard-Miceli & Criswell, 2012; Rosenblum et al., 2015). Hypomethylation of genes such as *CD40LG*, *CD70*, *HLA-DRB1*, *STAT1* (Miller et al., 2019), *IRF5* (Song et al., 2020), *IFIT2* (Siddiqi et al., 2021), *ITGAL* (Matatiele et al., 2015), *CD5* (Hurtado et al., 2020), *HRES1* (Hurtado et al., 2020), *LCN2* (Xiao et al., 2022), *IFNGR2* (Liu et al., 2022), *IFI44L* (Salesi et al., 2022), *USP18* (Wardowska, 2020), and *MMP14* (Chen et al., 2017) has been documented in SLE; *IL6* (Tang et al., 2014) and *CD40LG* (Zhao et al., 2022) in RA; and *HLA-DQB1*, *RFXAP* (Cerna, 2019), *NFKB1A* (Zhang et al., 2021), and *GAD2* (Dashti et al., 2022) in T1DM. Additionally, hypermethylation of *FOXP3* (Noori-Zadeh et al., 2017), *PTPN6* (Celarain & Tomas-Roig, 2020), and *TNF* (Bingen et al., 2022) in MS; *CD6* (Zhang et al., 2021) in T1DM; and *TNFRSF25* (Brandt et al., 2019) in RA have been associated with increased disease incidence. FOXP3 is essential for the induction of regulatory T cells and its deficiency leads to persistent immune hyperactivity. Utilizing the dCas9-SUNTAG-TET1 system (DNA demethylase complex), which targets *FOXP3*, a 20-30% reduction in T-cell proliferation was observed (Jeffries, 2018).

Aberrations in histone modifications have been documented in autoimmune disorders, including hypoacetylation of H3 and H4, hypomethylation of H3K9, increased acetylation of H3K18, methylation of H3K4, and hyperacetylation of H4 in T cells, particularly in SLE (Zhan et al., 2016). Furthermore, increased deacetylation of histone H3 and acetylation of H3K9 have been observed in MS and T1DM (Bingen et al., 2022; Miao et al., 2012; Pedre et al., 2011). These findings reinforce the mechanistic richness of epigenetic regulation in autoimmunity and support the translational potential of targeted editing.

Collectively, these findings illustrate the cross-disciplinary potential of epigenetic editing tools that have demonstrated promising applications in cancer, neurological disorders, and autoimmune diseases.

Cardiovascular diseases

Cardiovascular diseases (CVDs) encompass pathological conditions affecting the heart, blood vessels, or both, with clinical manifestations, including ischemia, hypertension, angina, myocardial infarction, and stroke. CVDs remain a leading cause of mortality and represent a significant global health burden, with an estimated prevalence ranging from 40% to 80%, depending on the region and age group (McPherson &

Tybjaerg-Hansen, 2016). Increasing evidence supports a strong link between epigenetic dysregulation and the development of CVDs, often in conjunction with gene–environment interactions. According to the molecular mechanisms:

DNA methylation: miRNA-217 is upregulated during cardiac hypertrophy, leading to a reduction in the functionality of histone methylation enzymes (Sum & Brewer, 2023). Hypomethylation of *EGFR* and *AMOTL2* and hypermethylation of *PECAM1* and *ARHGAP24* have been reported (Ordovas & Smith, 2010; Udali et al., 2013). In vascular pathologies, hypermethylation of *ESR1* and *MCT3*, along with hypomethylation of the *ALOX15* promoter (a lipid peroxidation enzyme), contributes to atherosclerosis progression (Li et al., 2016).

Histone modifications: Under hyperglycemic conditions, such as in diabetic patients, HATs facilitate the addition of acetyl groups to histones, activating nuclear factor kappa-light-chain-enhancer of activated B cells (*NFKB1*), and subsequently inducing acetylation of the p65 subunit, which in turn induces proinflammatory mediators, including *TNF* and *PTGS2* (Friso et al., 2008). Histone modifications also affect endothelial function; for instance, hyperacetylation of H3K9 and H4K12 and di-/tri-methylation of H3K4 have been associated with reduced *NOS3* expression by altering chromatin accessibility at the *NOS3* promoter (Fang et al., 2021).

Epigenetic editing tools: These platforms, particularly those utilizing CRISPR-based methodologies, have the potential to precisely manipulate target gene expression (Baccarelli & Ordovas, 2023). The expression of proprotein convertase subtilisin/kexin type 9 (*PCSK9*) is positively associated with circulating levels of low-density lipoprotein cholesterol (Porcheron et al., 2025). Whittaker et al. (2023) employed the epigenome editing tool CRISPRoff to knock down *PCSK9* in HuH hepatoma cells. This discovery offers novel insights into modifying the epigenetic status of *PCSK9* as a promising therapeutic strategy for CVD. Conversely, CRISPRa systems, such as dCas9-p300, have been used to activate protective genes, such as *NOS3*, enhancing endothelial nitric oxide production and vascular resilience.

Despite these promising developments, several translational challenges remain. Efficient delivery to target tissues, such as hepatocytes and endothelial cells, is critical, and current strategies include AAVs, lipid nanoparticles (LNPs), and mRNA-based platforms. Furthermore, the long-term stability of epigenetic modifications and their *in vivo* specificity are active areas of investigation, with ongoing efforts to engineer high-fidelity dCas9 variants and optimize delivery systems.

Collectively, these findings highlight the transformative potential of epigenetic editing in cardiovascular medicine. By integrating molecular insights with emerging therapeutic tools, this approach offers a compelling path towards precision interventions for complex multifactorial diseases.

Precise epigenetic editing tools vs. classical epigenetic drugs

Classical epigenetic drugs, defined as small-molecule inhibitors targeting enzymes that modify chromatin structure or DNA methylation, have distinct advantages over conventional therapies, such as radiotherapy, chemotherapy, and immunotherapy (Mabe et al., 2024). These pharmacological agents specifically target aberrant epigenetic characteristics in various diseases with the objective of restoring normal cellular function or enhancing immune system recognition (Qin et al., 2024). Additionally, they have the potential to overcome drug resistance, particularly in cancer cells (Xu et al., 2024). Several epigenetic drugs, including DNMT, HDAC, IDH, and EZH2 inhibitors, have been approved for commercial distribution. These inhibitors have been reviewed previously (Dai et al., 2024).

Despite their therapeutic potential, clinical applications are constrained by limitations, including neurotoxic effects (e.g., fatigue, confusion, and peripheral neuropathy), lack of target specificity, and poorly understood off-target mechanisms (Martinez-Iglesias et al., 2023; Shukla & Tekwani, 2020). Among the FDA-approved HDAC inhibitors, vorinostat (Grant et al., 2007), romidepsin (Bertino & Otterson, 2011), belinostat (Poole, 2014), and panobinostat (San-Miguel et al., 2014) have demonstrated efficacy in the treatment of hematological malignancies such as cutaneous T-cell lymphoma and multiple myeloma. These agents exert broad inhibitory effects on nearly all HDAC isoforms. However, this nonselective inhibition contributes to a wide array of side effects, including gastrointestinal distress, thrombocytopenia, and cardiac toxicity, which limits their broader clinical use (Shah, 2019).

As the understanding of HDAC isoform-specific functions advances, there is growing interest in the development of selective HDAC inhibitors with improved tolerability (Ho et al., 2020). However, HDAC5/6/7/8/10 lacks strong evidence of direct involvement in histone deacetylation, which complicates their validation as therapeutic targets. This uncertainty hinders rational drug design and contributes to developmental bottlenecks in next-generation HDAC inhibitors (Adhikari et al., 2021).

CRISPR-based epigenetic editing tools have emerged as promising alternatives for addressing the need for greater specificity and control. These systems demonstrate remarkable precision, enabling targeted modulation of gene expression at designated genomic loci while minimizing off-target effects. Their

programmability for multiplexing and potential reversibility present a sophisticated and controllable therapeutic strategy (Fadul et al., 2023).

CRISPR-based editing systems frequently target the acetylation of H3K27 residues. This is achieved by creating a nuclease-deficient dCas9 protein fused to the catalytic domains of acetyltransferases, such as p300, allowing the modulation of genes regulated by both proximal and distal enhancers (Gao & Liang, 2018). Studies have demonstrated that dCas9-p300 fusion proteins can activate endogenous genes, offering a powerful tool for enhancer interrogation.

To investigate enhancer function more comprehensively, dual-effector systems, known as enCRISPRa and enCRISPRi, were developed. The enCRISPRa system integrates the acetylation-writing domain p300 with the transcriptional activator VP64 to stimulate enhancer activity, whereas enCRISPRi combines the LSD1 lysine demethylase domain with a KRAB transcriptional repressor to disrupt enhancer function (Li et al., 2020b). These systems are valuable for functional genomics and therapeutic modulation, enabling precise control of gene regulatory elements.

CRISPR/Cas9-based HDAC fusion proteins have also been engineered for transcriptional repression. For example, the dCas9-HDAC3 fusion system has been shown to repress the transcription of endogenous promoters (Kwon et al., 2017). Targeted editing of the epigenome has transformed our capacity to explore essential biological processes and to modify cellular states. After extensive tool refinement and proof-of-concept studies, epigenetic editing has approached clinical translation, offering a novel strategy for treating diseases with limited therapeutic options.

In a recent study, Cappelluti et al. (2024) explored *PCSK9*, which is expressed in liver cells and regulates cholesterol levels. By evaluating various editor designs *in vitro*, they identified a zinc finger–based gene repressor as the most effective DNA-binding platform for silencing the murine *PCSK9* gene. A single dose of LNPs containing the mRNA of the editors led to a nearly 50% reduction in circulating PCSK9 levels for almost a year in mice. Silencing of *PCSK9* and associated epigenetic repressive marks persisted even after induced liver regeneration, supporting the heritability of the newly established epigenetic state. Furthermore, Tremblay et al. (2025) demonstrated that delivering the RNA form of the dCas9-KRAB editor encapsulated in LNPs to cynomolgus monkeys resulted in a ~90% reduction in circulating PCSK9 protein and approximately 70% decrease in low-density lipoprotein cholesterol for at least one year. Although these findings are promising, translational challenges remain, including the potential immunogenicity of RNA/protein editors and delivery efficiency to target tissues. These studies will pave the way for *in vivo* therapies based on epigenetic editing.

Although current platforms excel at gene repression, there is an urgent need for tools capable of achieving durable gene activation. Addressing this gap is essential to expand the therapeutic scope of epigenetic editing.

Limitations and challenges

The epigenome also plays a crucial role in cellular development. It regulates gene expression and influences the emergence of various phenotypes, making it central to the understanding of disease mechanisms and therapeutic innovation. Owing to the pivotal role of the epigenome, efforts to modify and manipulate it to understand gene function in phenotype expression, cell development, cell reprogramming, and the treatment of epigenetic-related diseases have become a prominent focus of research recently. Despite progress in this field, epigenomic manipulation remains in its nascent stages, particularly in terms of clinical applications, tool precision, and delivery strategies. For instance, while pan-HDAC inhibitors have demonstrated therapeutic potential, they frequently induce widespread side effects and toxicities owing to their broad, nonselective inhibition, including fatigue, gastrointestinal distress, and hematologic toxicity. Furthermore, although novel selective HDAC inhibitors are being developed as more tolerable alternatives, they continue to encounter significant developmental challenges, and although evidence remains limited, several promising candidates are under active investigation (Dai et al., 2024).

The advent of genomic editing tools has markedly enhanced the capacity of epigenetic editing, thereby paving the way for more precise and targeted therapeutic strategies. The wild-type CRISPR/Cas9 system encounters a substantial challenge owing to off-target effects, prompting the development of high-fidelity CRISPR-based technologies (Bayat et al., 2024a; Shams et al., 2022). Off-target effects are especially problematic in epigenetic editing because they can result in unintended and potentially persistent changes in chromatin states. In this context, off-target issues emerge from (i) high concentrations of effector domains, which may lead to non-specific activity (Policarpi et al., 2024), (ii) the tendency of epigenetic marks to spread beyond the intended locus (Lensch et al., 2022), and (iii) partial homology between sgRNAs and non-target sequences, causing unintended binding (Fadul et al., 2023; Tadic et al., 2019).

Although sgRNA engineering and enhanced Cas9 fidelity have been designed to address these concerns, significant gaps remain, particularly in the need for comprehensive genome-wide assessments of transcriptional and chromatin changes. Techniques, such as ChIP-seq, ATAC-seq, and RNA-seq, are essential for evaluating off-target effects and validating safety profiles for clinical translation (Nunez et al., 2021; Shi et al., 2025).

The efficacy of epigenetic editing, as measured by alterations in gene expression, can reach 1,000-fold

modulation, including both gene activation and repression, depending on the effector used. CRISPRi exhibits strong gene silencing capabilities, offering a potent alternative to gene knockout in high-throughput screening applications, whereas DNA methylation ensures the stable maintenance of these repressive states across cell divisions. However, the overall potency of both gene activation and repression is subject to significant variability (Karbassi et al., 2024). This variability is influenced by multiple factors, including (i) the specific cell type and targeting context, (ii) the precise design and positioning of the sgRNA (chromatin accessibility, sequence specificity, and local epigenetic context), and (iii) the type and expression level of epigenetic editing tools (Roth et al., 2024). Consequently, achieving the desired efficiency in epigenetic editing often requires extensive and iterative optimization. A major ongoing challenge is ensuring the persistence of therapeutic effects, as epigenetic markers in proliferating cells may be diluted during successive cell divisions. Addressing this critical issue necessitates the development of novel strategies to actively induce endogenous maintenance mechanisms. As a result, it enables the "self-sustainability" of epigenetic modifications and replicates the inherent stability of the natural epigenome. The persistence of epigenetic modifications is maintained by enzymes, such as DNMTs and histone-modifying complexes. Clinically, the persistence or dilution of epigenetic marks is particularly relevant for proliferative diseases, such as cancer, where sustained repression or activation is essential for therapeutic efficacy.

Additionally, effective delivery vehicles are crucial for direct administration of epigenetic editing tools to target cells. The current landscape of delivery vehicles for epigenetic editing tools, including viral, LNP, and other non-viral approaches, has significant limitations that impede reliable clinical translation. Viral vectors, such as adeno-associated viruses, adenoviruses, and lentiviruses, offer certain advantages in specific contexts. However, challenges such as limited packaging capacity, persistent transgene expression, high immunogenicity, potential genomic integration, and inadequate cell type specificity restrict their capacities. LNPs have emerged as promising platforms owing to their transient cargo delivery and reduced immunogenicity. However, liver tropism and an inability to efficiently transfect the central CNS or various other specific cell types and tissues have pronounced limitations. Ongoing research is exploring surface modifications and ligand conjugation to redirect LNPs towards nonhepatic tissues (Jallow et al., 2025). Similarly, other non-viral methods, such as direct ribonucleoprotein (RNP) delivery, suffer from low transfection efficiencies and technical difficulties, especially in CNS tissues, where cellular uptake and nuclear localization are particularly challenging. Furthermore, virus-like particles (VLPs), although theoretically versatile, currently face challenges related to the engineering of epigenetic editing tools, insufficient RNP lifetime, sgRNA packaging, limited *in vivo* efficiency, and significant obstacles in scalability and standardization.

Exosomes, which are natural nanoparticles that facilitate cellular communication, have recently demonstrated promising potential for delivering therapeutic cargo to target cells (Tenchov et al., 2022). These advances have helped overcome prior limitations in tissue specificity and immunogenicity. Recently, Ma et al. (2025) used surface-modified bone marrow mesenchymal stem cell-derived exosomes to successfully manipulate the epigenetics of aging nucleus pulposus cells to restore a youthful epigenetic state. Furthermore, Shrivastava et al. (2021) introduced a novel therapeutic approach by engineering a ZF fused to DNMT3A to silence the HIV-1 promoter. They encapsulated RNAs encoding this repressor protein within exosomes for delivery into humanized NSG mouse models. These engineered exosomes effectively inhibited viral expression by inducing DNA methylation of HIV-1, thereby demonstrating the potential of an exosome-based systemic delivery system. These achievements, along with a growing body of published evidence, indicate that exosomes represent a promising vehicle for targeted delivery of epigenetic editing tools.

Future research may include engineered exosomes with enhanced targeting capabilities, improved LNP formulations for broader tissue access, and combinational delivery strategies that integrate multiple platforms. In conclusion, despite ongoing advancements, a universally safe, efficient, and targeted delivery system for epigenetic editing remains a laborious challenge.

Conclusion and Future Directions

Epigenome editing is evolving rapidly from a foundational research tool to a transformative therapeutic strategy. The field has undergone a significant transformation from a tool primarily used for fundamental biological research to a highly promising therapeutic approach. These technologies have deepened our understanding of how epigenetic marks affect gene expression and present considerable potential for identifying novel therapeutic targets and precisely modifying cellular states. This evolution positions epigenome editing as a pioneering approach for the treatment of diseases, particularly those with limited existing interventions, and emphasizes the need to develop tools capable of inducing persistent gene activation along with established silencing capabilities.

Despite these substantial advancements, several critical challenges must be addressed to fully realize the clinical potential of epigenome editing. A primary concern involves improving the specificity of these tools to mitigate off-target effects, necessitating continued exploration of more precise CRISPR platforms. Additionally, ensuring the efficient and safe *in vivo*

delivery of epigenome-editing components remains a major obstacle for clinical translation. Progress has been made with viral vectors, such as AAVs, which offer high transduction efficiency but pose immunogenicity risks, and non-viral methods, including LNPs (Woodward et al., 2024), which provide lower immunogenicity and scalable manufacturing but face limitations in tissue targeting. Moreover, cell-penetrating peptides (de Morais et al., 2024) and gold nanoparticles (Cavazza et al., 2025), universally safe and effective delivery systems, remain unmet needs. Future research should prioritize the development of innovative delivery technologies that offer improved tissue specificity, reduced immunogenicity, and enhanced cargo packaging capacity.

To envision the future of research, a pivotal theoretical framework involves utilizing our expanding comprehension of epigenetic mechanisms to develop highly selective and targeted pharmacological agents. These can precisely address the heterogeneity of epigenetic hallmarks of various diseases. This endeavor will necessitate rigorous *in vitro* and *in vivo* validation of novel drug candidates, particularly those identified using virtual screening methodologies. The recent identification of eukaryotic programmable RNA-guided endonucleases, such as Fanzor (Saito et al., 2023) and OMEGA–IscB (Kannan et al., 2025), exemplifies the ongoing efforts to discover novel and efficient genome editing tools. Continued exploration of diverse biological systems is likely to yield new classes of highly precise and adaptable epigenetic editors. This leads to a compelling hypothesis: sustained investigation of the vast natural repertoire of biological mechanisms will unlock even more diverse and effective tools for precision epigenome engineering.

Future directions for epigenome editing should focus on several critical areas. First, increased emphasis on developing self-sustaining epigenetic edits emulated the endurance of natural epigenomic stability. This involves creating systems that actively induce endogenous maintenance mechanisms, thereby preventing the dilution of epigenetic markers during cell division and ensuring long-term therapeutic effects. Second, significant attention will be directed towards comprehensively assessing the therapeutic potential of epigenetic drugs in advanced clinical trials, moving beyond preclinical studies to validate their efficacy and safety in human patients. Third, integrating innovative drug discovery technologies is crucial for accelerating the development of novel epigenetic-based drugs. Finally, this field is poised to explore the synergistic potential of combining epigenetic-targeted agents with traditional therapeutic approaches to achieve enhanced clinical outcomes.

By harnessing the synergy between epigenetic editing and delivery sciences, which encompass viral and non-viral vectors, nanoparticle engineering, and tissue-specific targeting strategies, genome engineers have been positioned to revolutionize human health. This groundbreaking convergence promises to be used in an era of highly personalized and remarkably effective epigenetic therapeutic strategies. This powerful approach could lead to treatments that are precisely customized for each person, improving results, and changing the way healthcare works.

Author contributions

MM: Investigation, Methodology, Writing – Original Draft preparation; NH: Conceptualization, Methodology, Writing –Original Draft preparation; SHT, FSH and YT: Investigation, Writing – Review & Editing; AR: Funding Acquisition, Project Administration, Writing – Review & Editing

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Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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Table 1. Diversity of epigenetic modifications, critical modification sites, modifying enzymes, and their association with specific diseases.

Type of modification	Modification sites	Modifying enzymes	Relevant diseases	References
Acetylation (Active condition)	H3K4/H3K9/H314/H3K18/H3K23/H3K27/H3K36/H3K56 H4K5/H4K8/H4K12/H4K16/H4K20	HATs	Cancer, Infectious diseases, Neurological disorders and Autoimmune diseases	(Alaskhar Alhamwe et al., 2018; Saito et al., 2014; Shukla & Tekwani, 2020)
Methylation (Active condition)	H3K4/H3K36/H3K79(Trimethylated) H3K9/H3K27 H4K20	HMTs and KMTs	Cancer, Infectious diseases, Neurological disorders and Autoimmune diseases	(Alaskhar Alhamwe et al., 2018; Jin & Liu, 2018; Lakshminarasimhan & Liang, 2016; Rasmi et al., 2023)
Methylation (Inactive condition)	H3R2/ H3K9/H3K27 (Dimethylated)	KDMs		(Jin & Liu, 2018; Lakshminarasimhan & Liang, 2016; Rasmi et al., 2023; Vukic & Daxinger, 2019)
Phosphorylation (Active condition)	H3S10/H3Y41/H3T45 H4S1	PKs	Diabetic kidney disease	(Alghamdi et al., 2018; Pang et al., 2022)
Ubiquitination	H2AK119 H2BK120	Histone ubiquitin transferase	Cancer	(Espinosa, 2008)
Sumoylation (Inactive condition)	H4K5/H4K8/H4K12/H4K16/H4K20	E1-activating enzyme E2-conjugating enzyme E3 ligases	Cancer	(Zhao et al., 2020)
ADP-ribosylation (Active condition)	H2BE18/H2BE19	PARP	Cancer, Infectious disease, Neurological disorders and Autoimmune diseases	(McGurk et al., 2019; Palazzo et al., 2019)
DNA methylation	Cytosine at carbon- 5 in CpG islands	DNMT	Cancer, Infectious disease, Neurological disorders and Autoimmune diseases	(Qin et al., 2021; Richardson, 2003; Younesian et al., 2022)
Non-coding RNA	N6-methyladenosine m^6A/ N1-methyladenosine (m^1A)/ inosine (I)/ 5-methylcytidine (m^5C)/ pseudouridine (Ψ)	ADARs, METTL3, and METTL14	Cancer, Neurological disorders and Autoimmune diseases	(Kazimierczyk & Wrzesinski, 2021; Lodde et al., 2020; Salvatori et al., 2020; Yang et al., 2020)

Table 2. Various epigenetics editing platforms and their effectors.

Epigenetics editing platform	Advantages and Disadvantages	Gene activation effectors	Gene repression effectors	References
ZFN	-Combined using modular assembly -High frequency of off-target -Able to bind condensed and hypermethylated DNA - For a new target site, the DNA-binding domain must be custom-designed - Can be delivered even in vectors with limited packaging capacity	VP16 / VP64 / p65 / TET enzymes p300	DNMTs (DNMT3A and DNMT3L) HMTs (G9A and SUV39H1) KRAB	(Laufer & Singh, 2015; Ueda et al., 2023)
TALEs	-Can be assembled using golden gate cloning methods, FLASH assembly, or iterative capped assembly -Low off-target effect -Sensitive to hypermethylated DNA -For each new target site must be custom-designed and built for each new target, repetitive structure can cause cloning problems -Need vectors with high capacity (high mutation and recombination rate in lentiviral delivery)	VP16 / VP64 / TET1	KRAB mSin interaction domain (SID) LSD1	(Laufer & Singh, 2015; Lee et al., 2016; Ueda et al., 2023)
CRISPR/Cas	-different rate of off-target effects (High-fidelity forms reduced up to undetectable levels) -Can bind condensed and hypermethylated DNAs -Simple cloning and multiple editing -Needs high capacity vector for delivery (smaller variants introduced; can be delivered in RNA and RNP forms)	VP16 / VP64 / VP160 /VP192 / VPR (VP64, p65 / Rta) TET1 p300 PRDM9 DOT1L	KRAB LSD1 G9A DNMT3A / DNMT3L	(Brezgin et al., 2019; Laufer & Singh, 2015; Syding et al., 2020)

Table 3. Applications of epigenetic editing tools based on CRISPR/Cas9 technology.

Application	Effector	Main findings	Cell types	Targeted gene	References
Discovering the role of effectors	SMYD3	Confirmed SMYD3 role in depositing H3K4me3	HEK 293	*FNBP1*	(Kim et al., 2015)
	LSD1	Highlighted the specificity of the LSD1-induced enhancer deactivation	mouse embryonic stem cells (mESCs)	*TBX3*	(Kearns et al., 2015)
	BAF	Demonstrated BAF context-dependent activity in controlling gene activation	mouse embryonic stem cells (mESCs)	*Nkx2*	(Braun et al., 2017)
Cell differentiation and reprogramming	VP64	H3K27ac converts fibroblasts to neuronal cells	mouse embryonic fibroblasts (mEFs)	*Brn2 Ascl1 Myt1l*	(Black et al., 2016)
	p300	H3K27ac modulation for cell reprogramming to pluripotency	mouse embryonic fibroblasts (mEFs)	*Oct4* *Sox2*	(Liu et al., 2018)
Therapeutic epigenetic editing (*in vitro*)	TET1	Restoration of *BRCA1* expression	HeLa MCF7	*BRCA1*	(Choudhury et al., 2016)
	VPR	Reactivation of tumor suppressor genes	H157 MCF7 SUM159	*MASPIN REPRIMO*	(Garcia-Bloj et al., 2016)

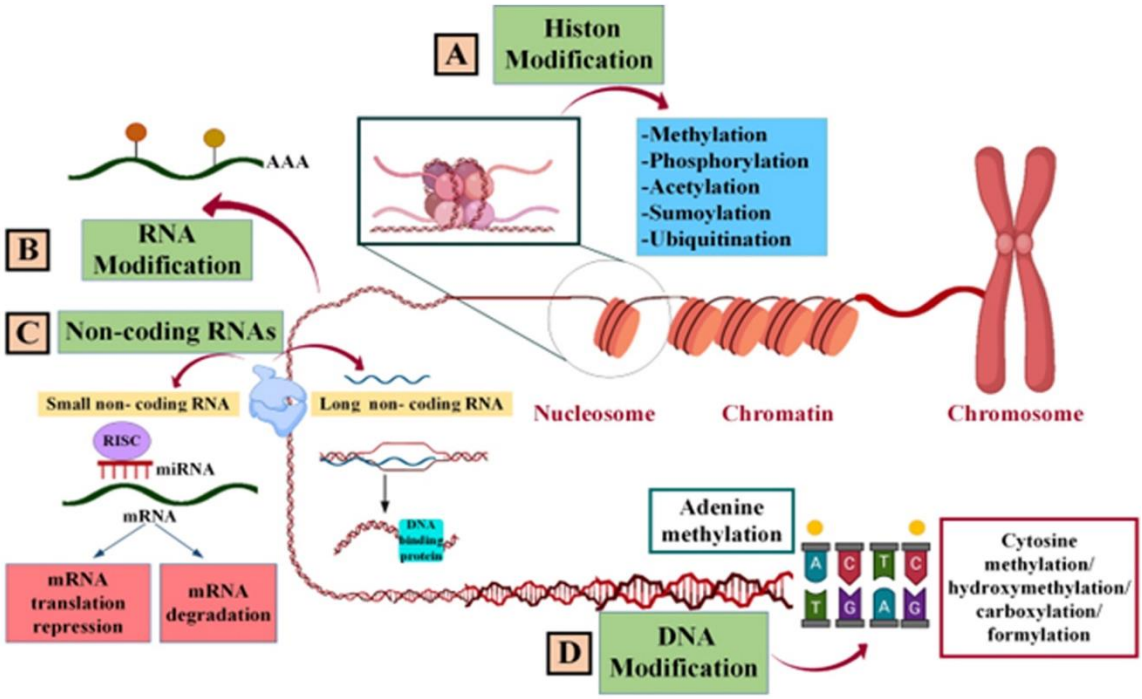

Figure 1. Epigenetic Modifications in Gene Expression Regulation. A) Histone modifications, recognized as post-translational DNA modifications, typically occur via methylation or acetylation. These modifications influence gene expression by either relaxing or compacting nucleosomes, thereby activating or repressing transcription. **(B**) Over 160 known types of RNA nucleotides can undergo chemical modifications, including N6-methyladenosine (m6A). **C**) Non-coding RNA pathways encompassing both small and long ncRNA species play a crucial role in transcriptional regulation and are generally regarded as epigenetic mechanisms. LncRNAs are associated with various complexes and can either activate or repress transcription. **D**) DNA can be chemically modified at cytosine and adenine residues. Cytosine modifications include methylation, formylation, hydroxymethylation, and carboxylation, whereas adenine is modified through methylation.

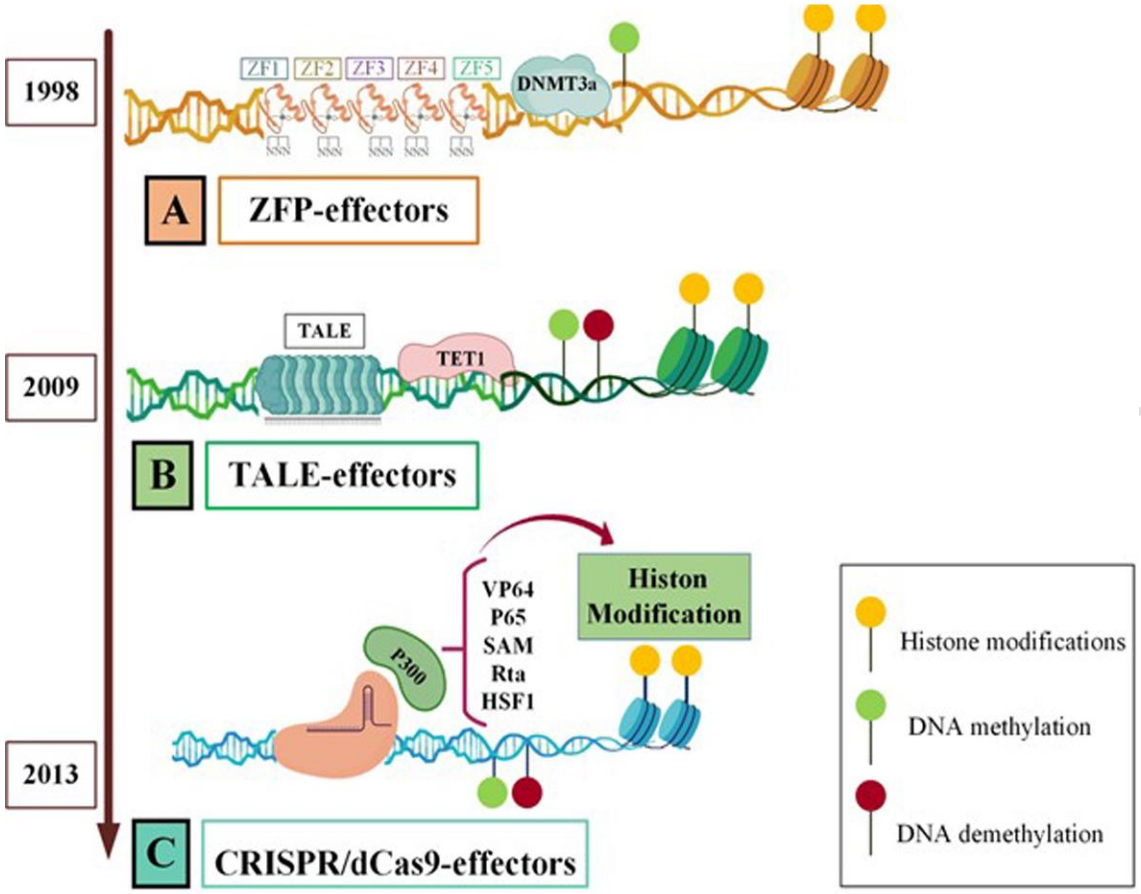

Figure 2. Epigenetic Editing. A) In zinc finger arrays, each module predominantly recognizes three base pairs of DNA, facilitating targeted DNA methylation by DNMT3a. **B**) In the TALE effectors, each repeat unit recognizes a single base pair, enabling targeted DNA methylation by TET1. **C**) In the CRISPR/dCas9 system, one strand of the target site is identified through Watson-Crick base pairing with a bound guide RNA, and sgRNA facilitates complementary targeted histone modification by P300 as well as interactions with other molecules such as VP64, P65, SAM, Rta, and HSF1.