

REVIEW

# Epigenome editing: emerging tools, therapeutic applications, and challenges in human disease treatment

Maryam Mirahmadi<sup>1,2</sup> , Nader Hashemi<sup>3,4</sup> , Sayed Hassan Tabatabaee<sup>5</sup> ,  
Forough Shams<sup>4</sup> , Yong Teng<sup>6,7</sup> , Azam Rahimpour<sup>3,4\*</sup> 

<sup>1</sup>Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, 14115111, Tehran, Iran

<sup>2</sup>Department of Exomine, PardisGene company, 14115111, Tehran, Iran

<sup>3</sup>Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, 1968917313, Tehran, Iran

<sup>4</sup>Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, 1968917313, Tehran, Iran

<sup>5</sup>Department of Life Science Engineering, Faculty of New Sciences and Technology, University of Tehran, 1417935840, Tehran, Iran

<sup>6</sup>Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA 30322, USA

<sup>7</sup>Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30322, USA

## How to cite:

Mirahmadi, M., Hashemi, N., Tabatabaee, S. H., Shams, F., Teng, Y., Rahimpour, A. (2026). Epigenome Editing: Emerging Tools, Therapeutic Applications, and Challenges in Human Disease Treatment. *Biotech Studies* 35, 1830114.

<http://doi.org/10.38042/biotechstudies.1830114>

## Article History

Received 11 February 2025

Accepted 20 October 2025

First Online 10 November 2025

## Corresponding Author

Tel.: +98 218 866 6140

E-mail: rahimpour@sbmu.ac.ir

## Keywords

Epigenetic

Genome editing

CRISPR/Cas

TALEN

ZFN

## Copyright

This is an open-access article

distributed under the terms of the

[Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/)

[International License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/).

## 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.

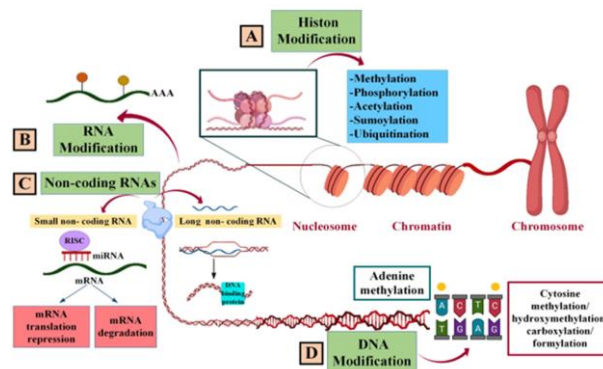
Aberrant epigenetic modifications have been implicated in numerous diseases, including cardiovascular diseases, neurological disorders, and cancer (Robusti et al., 2022). For example, global hypomethylation and gene-specific hypermethylation are common in cancer, whereas atypical histone acetylation 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).



**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.

### 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

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

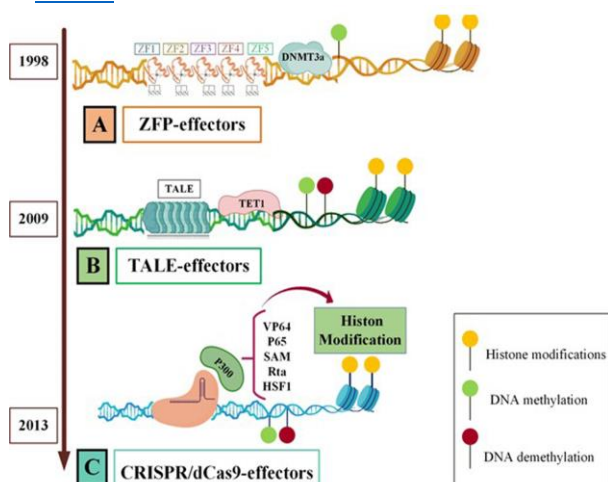
Type of modification	Modification sites	Modifying enzymes	Relevant diseases	References
Acetylation (Active condition)	H3K4/H3K9/H314/H3K18/H3K23/ H3K27/H3K36/H3K56/H4K5/H4K8 /H4K12/H4K16/H4K20	HATs	Cancer, Infectious diseases, Neurological disorders and Autoimmune diseases	(Alaskhar Alhamwe et al., 2018; 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 <sup>6</sup> A)/ N1-methyladenosine (m <sup>1</sup> A)/ inosine (I)/ 5-methylcytidine (m <sup>5</sup> C)/ 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)

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.



**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 targeted with other molecules such as VP64, P65, SAM, Rta, and HSF1.

### 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  $\alpha$ -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 II 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

**Table 2.** Various epigenetics editing platforms and their effectors

Epigenetics editing platform	Advantages and Disadvantages	Gene activation effectors	Gene repression effectors	References
ZFN	<ul style="list-style-type: none"> <li>-Combined using modular assembly</li> <li>-High frequency of off-target</li> <li>-Able to bind condensed and hypermethylated DNA</li> <li>-For a new target site, the DNA-binding domain must be custom-designed</li> <li>-Can be delivered even in vectors with limited packaging capacity</li> </ul>	VP16 / VP64 / p65 / TET enzymes p300	DNMTs (DNMT3A and DNMT3L) HMTs (G9A and SUV39H1) KRAB	<a href="#">(Laufer &amp; Singh, 2015; Ueda et al., 2023)</a>
TALEs	<ul style="list-style-type: none"> <li>-Can be assembled using golden gate cloning methods, FLASH assembly, or iterative capped assembly</li> <li>-Low off-target effect</li> <li>-Sensitive to hypermethylated DNA</li> <li>-For each new target site must be custom-designed and built for each new target, repetitive structure can cause cloning problems</li> <li>-Need vectors with high capacity (high mutation and recombination rate in lentiviral delivery)</li> </ul>	VP16 / VP64 / TET1	KRAB mSin interaction domain (SID) LSD1	<a href="#">(Laufer &amp; Singh, 2015; Lee et al., 2016; Ueda et al., 2023)</a>
CRISPR/Cas	<ul style="list-style-type: none"> <li>-Different rate of off-target effects (High-fidelity forms reduced up to undetectable levels)</li> <li>-Can bind condensed and hypermethylated DNAs</li> <li>-Simple cloning and multiple editing</li> <li>-Needs high-capacity vector for delivery (smaller variants introduced; can be delivered in RNA and RNP forms)</li> </ul>	VP16 / VP64 / VP160 / VP192 / VPR (VP64, p65 / Rta) TET1 p300 PRDM9 DOT1L	KRAB LSD1 G9A DNMT3A / DNMT3L	<a href="#">(Brezgin et al., 2019; Laufer &amp; Singh, 2015; Syding et al., 2020)</a>

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 transactivating 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 remodelling ([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).

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

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

### Funding Information

This work was supported by Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant Number 29763, Ethics Code: IR.SBMU.RETECH.REC.1400.655) which was awarded to AR.

### Acknowledgements

The authors wish to thank Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, for their support.

### References

- Adhikari, N., Jha, T., & Ghosh, B. (2021). Dissecting Histone Deacetylase 3 in Multiple Disease Conditions: Selective Inhibition as a Promising Therapeutic Strategy. *Journal of Medicinal Chemistry*, 64(13), 8827-8869. <https://doi.org/10.1021/acs.jmedchem.0c01676>
- Alaskhar Alhamwe, B., Khalaila, R., Wolf, J., von Bulow, V., Harb, H., Alhamdan, F., Hii, C. S., Prescott, S. L., Ferrante, A., Renz, H., Garn, H., & Potaczek, D. P. (2018). Histone modifications and their role in epigenetics of atopy and allergic diseases. *Allergy, Asthma, and Clinical Immunology*, 14, 39. <https://doi.org/10.1186/s13223-018-0259-4>
- Alghamdi, T. A., Batchu, S. N., Hadden, M. J., Yerra, V. G., Liu, Y., Bowskill, B. B., Advani, S. L., Geldenhuys, L., Siddiqi, F. S., Majumder, S., & Advani, A. (2018). Histone H3 Serine 10 Phosphorylation Facilitates Endothelial Activation in Diabetic Kidney Disease. *Diabetes*, 67(12), 2668-2681. <https://doi.org/10.2337/db18-0124>
- Altinbay, M., Wang, J., Chen, J., Schafer, D., Sprang, M., Blagojevic, B., Wolf, S., Andrade-Navarro, M. A., Dikic, I., Knapp, S., & Cheng, X. (2024). Chem-CRISPR/dCas9FCPF: a platform for chemically induced epigenome editing. *Nucleic Acids Research*, 52(19), 11587-11601. <https://doi.org/10.1093/nar/gkae798>
- Baccarelli, A. A., & Ordovas, J. (2023). Epigenetics of Early Cardiometabolic Disease: Mechanisms and Precision Medicine. *Circulation Research*, 132(12), 1648-1662. <https://doi.org/10.1161/CIRCRESAHA.123.322135>
- Bayat, H., Farahmand, F., Tabatabaee, S. H., Shams, F., Mohammadian, O., Pourmaleki, E., & Rahimpour, A. (2024a). Evaluation of the paired-Cas9 nickase and RNA-guided FokI genome editing tools in precise integration of an anti-CD52 bicistronic monoclonal antibody expression construct at Chinese hamster ovary cells 18S rDNA locus. *Protein Expression and Purification*, 217, 106445. <https://doi.org/10.1016/j.pep.2024.106445>
- Bayat, H., Mirahmadi, M., Azarshin, Z., Ohadi, H., Delbari, A., & Ohadi, M. (2024b). CRISPR/Cas9-mediated deletion of a GA-repeat in human GPM6B leads to disruption of neural cell differentiation from NT2 cells. *Scientific Reports*, 14(1), 2136. <https://doi.org/10.1038/s41598-024-52675-3>
- Bayat, H., Modarressi, M. H., & Rahimpour, A. (2018). The Conspicuity of CRISPR-Cpf1 System as a Significant Breakthrough in Genome Editing. *Current Microbiology*, 75(1), 107-115. <https://doi.org/10.1007/s00284-017-1406-8>
- Bayat, H., Omid, M., Rajabibazl, M., Sabri, S., & Rahimpour, A. (2017). The CRISPR Growth Spurt: from Bench to Clinic on Versatile Small RNAs. *Journal of Microbiology and Biotechnology*, 27(2), 207-218. <https://doi.org/10.4014/jmb.1607.07005>
- Becker, S., & Boch, J. (2021). TALE and TALEN genome editing technologies. *Gene and Genome Editing*, 2. <https://doi.org/10.1016/j.ggedit.2021.100007>
- Berdasco, M., & Esteller, M. (2013). Genetic syndromes caused by mutations in epigenetic genes. *Human Genetics*, 132(4), 359-383. <https://doi.org/10.1007/s00439-013-1271-x>
- Bertino, E. M., & Otterson, G. A. (2011). Romidepsin: a novel histone deacetylase inhibitor for cancer. *Expert Opinion on Investigational Drugs*, 20(8), 1151-1158. <https://doi.org/10.1517/13543784.2011.594437>
- Bingen, J. M., Clark, L. V., Band, M. R., Munzir, I., & Carrithers, M. D. (2022). Differential DNA methylation associated with multiple sclerosis and disease modifying treatments in an underrepresented minority population. *Frontiers in Genetics*, 13, 1058817. <https://doi.org/10.3389/fgene.2022.1058817>
- Black, J. B., Adler, A. F., Wang, H. G., D'ippolito, A. M., Hutchinson, H. A., Reddy, T. E., Pitt, G. S., Leong, K. W., &

- Gersbach, C. A. (2016). Targeted Epigenetic Remodeling of Endogenous Loci by CRISPR/Cas9-Based Transcriptional Activators Directly Converts Fibroblasts to Neuronal Cells. *Cell Stem Cell*, 19(3), 406-414. <https://doi.org/10.1016/j.stem.2016.07.001>
- Blum, K., Bowirrat, A., Baron, D., Elman, I., Makale, M. T., Cadet, J. L., Thanos, P. K., Hanna, C., Ahmed, R., Gondre-Lewis, M. C., Dennen, C. A., Braverman, E. R., Soni, D., Carney, P., Khalsa, J., Modestino, E. J., Barh, D., Bagchi, D., Badgaiyan, R. D.,...Gold, M. S. (2024). Identification of stress-induced epigenetic methylation onto dopamine D2 gene and neurological and behavioral consequences. *Gene & Protein in Disease*, 3(1). <https://doi.org/10.36922/gpd.1966>
- Bohnsack, J. P., Zhang, H., Wandling, G. M., He, D., Kyzar, E. J., Lasek, A. W., & Pandey, S. C. (2022). Targeted epigenomic editing ameliorates adult anxiety and excessive drinking after adolescent alcohol exposure. *Science Advances*, 8(18), eabn2748. <https://doi.org/10.1126/sciadv.abn2748>
- Brandt, B., Rashidiani, S., Ban, A., & Rauch, T. A. (2019). DNA Methylation-Governed Gene Expression in Autoimmune Arthritis. *International Journal of Molecular Sciences*, 20(22). <https://doi.org/10.3390/ijms20225646>
- Braun, S. M. G., Kirkland, J. G., Chory, E. J., Husmann, D., Calarco, J. P., & Crabtree, G. R. (2017). Rapid and reversible epigenome editing by endogenous chromatin regulators. *Nature Communication*, 8(1), 560. <https://doi.org/10.1038/s41467-017-00644-y>
- Brezgin, S., Kostyusheva, A., Kostyushev, D., & Chulanov, V. (2019). Dead Cas Systems: Types, Principles, and Applications. *International Journal of Molecular Sciences*, 20(23). <https://doi.org/10.3390/ijms20236041>
- Cappelluti, M. A., Mollica Poeta, V., Valsoni, S., Quarato, P., Merlin, S., Merelli, I., & Lombardo, A. (2024). Durable and efficient gene silencing in vivo by hit-and-run epigenome editing. *Nature*, 627(8003), 416-423. <https://doi.org/10.1038/s41586-024-07087-8>
- Castro-Munoz, L. J., Ulloa, E. V., Sahlgren, C., Lizano, M., De La Cruz-Hernandez, E., & Contreras-Paredes, A. (2023). Modulating epigenetic modifications for cancer therapy (Review). *Oncology Reports*, 49(3). <https://doi.org/10.3892/or.2023.8496>
- Cavazza, A., Molina-Estevéz, F. J., Reyes, A. P., Ronco, V., Naseem, A., Malensek, S., Pecan, P., Santini, A., Heredia, P., Aguilar-Gonzalez, A., Boulaiz, H., Ni, Q., Cortijo-Gutierrez, M., Pavlovic, K., Herrera, I., de la Cerda, B., Garcia-Tenorio, E. M., Richard, E., Granados-Principal, S.,...Benabdellah, K. (2025). Advanced delivery systems for gene editing: A comprehensive review from the GenE-HumDi COST Action Working Group. *Molecular Therapy Nucleic Acids*, 36(1), 102457. <https://doi.org/10.1016/j.omtn.2025.102457>
- Celarain, N., & Tomas-Roig, J. (2020). Aberrant DNA methylation profile exacerbates inflammation and neurodegeneration in multiple sclerosis patients. *Journal of Neuroinflammation*, 17(1), 21. <https://doi.org/10.1186/s12974-019-1667-1>
- Cerna, M. (2019). Epigenetic Regulation in Etiology of Type 1 Diabetes Mellitus. *International Journal of Molecular Sciences*, 21(1). <https://doi.org/10.3390/ijms21010036>
- Chandrasegaran, S. (2017). Recent advances in the use of ZFN-mediated gene editing for human gene therapy. *Cell & Gene Therapy Insights*, 3(1), 33-41. <https://doi.org/10.18609/cgti.2017.005>
- Chen, S. H., Lv, Q. L., Hu, L., Peng, M. J., Wang, G. H., & Sun, B. (2017). DNA methylation alterations in the pathogenesis of lupus. *Clinical and Experimental Immunology*, 187(2), 185-192. <https://doi.org/10.1111/cei.12877>
- Chen, Y., Ren, B., Yang, J., Wang, H., Yang, G., Xu, R., You, L., & Zhao, Y. (2020). The role of histone methylation in the development of digestive cancers: a potential direction for cancer management. *Signal Transduction and Targeted Therapy*, 5(1), 143. <https://doi.org/10.1038/s41392-020-00252-1>
- Choudhury, S. R., Cui, Y., Lubecka, K., Stefanska, B., & Irudayaraj, J. (2016). CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter. *Oncotarget*, 7(29), 46545-46556. <https://doi.org/10.18632/oncotarget.10234>
- Costa, P., Sales, S. L. A., Pinheiro, D. P., Pontes, L. Q., Maranhao, S. S., Pessoa, C. D. O., Furtado, G. P., & Furtado, C. L. M. (2023). Epigenetic reprogramming in cancer: From diagnosis to treatment. *Frontiers in Cell & Developmental Biology*, 11, 1116805. <https://doi.org/10.3389/fcell.2023.1116805>
- Dai, W., Qiao, X., Fang, Y., Guo, R., Bai, P., Liu, S., Li, T., Jiang, Y., Wei, S., Na, Z., Xiao, X., & Li, D. (2024). Epigenetics-targeted drugs: current paradigms and future challenges. *Signal Transduction and Targeted Therapy*, 9(1), 332. <https://doi.org/10.1038/s41392-024-02039-0>
- Dashti, M., Nizam, R., Hebbbar, P., Jacob, S., John, S. E., Channanath, A., Al-Kandari, H., Thanaraj, T. A., & Al-Mulla, F. (2022). Differentially methylated and expressed genes in familial type 1 diabetes. *Scientific Reports*, 12(1), 11045. <https://doi.org/10.1038/s41598-022-15304-5>
- de Moraes, C., Correia, E. M., Bonamino, M. H., & Vasconcelos, Z. F. M. (2024). Cell-Penetrating Peptides and CRISPR-Cas9: A Combined Strategy for Human Genetic Disease Therapy. *Human Gene Therapy*, 35(19-20), 781-797. <https://doi.org/10.1089/hum.2024.020>
- Dehshahri, A., Biagioni, A., Bayat, H., Lee, E. H. C., Hashemabadi, M., Fekri, H. S., Zarrabi, A., Mohammadinejad, R., & Kumar, A. P. (2021). Editing SOX Genes by CRISPR-Cas: Current Insights and Future Perspectives. *International Journal of Molecular Sciences*, 22(21). <https://doi.org/10.3390/ijms222111321>
- Dhar, G. A., Saha, S., Mitra, P., & Nag Chaudhuri, R. (2021). DNA methylation and regulation of gene expression: Guardian of our health. *Nucleus*, 64(3), 259-270. <https://doi.org/10.1007/s13237-021-00367-y>
- Eisenstein, M. (2012). Sangamo's lead zinc-finger therapy flops in diabetic neuropathy. *Nature Biotechnology*, 30(2), 121-123. <https://doi.org/10.1038/nbt0212-121a>
- Espinosa, J. M. (2008). Histone H2B ubiquitination: the cancer connection. *Genes & Development*, 22(20), 2743-2749. <https://doi.org/10.1101/gad.1732108>
- Fadul, S. M., Arshad, A., & Mehmood, R. (2023). CRISPR-based epigenome editing: mechanisms and applications. *Epigenomics*, 15(21), 1137-1155. <https://doi.org/10.2217/epi-2023-0281>
- Fang, Z., Wang, X., Sun, X., Hu, W., & Miao, Q. R. (2021). The Role of Histone Protein Acetylation in Regulating Endothelial Function. *Frontiers in Cell and Developmental Biology*, 9, 672447. <https://doi.org/10.3389/fcell.2021.672447>
- Friso, S., Pizzolo, F., Choi, S. W., Guarini, P., Castagna, A., Ravagnani, V., Carletto, A., Pattini, P., Corrocher, R., &

- Olivieri, O. (2008). Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. *Atherosclerosis*, 199(2), 323-327. <https://doi.org/10.1016/j.atherosclerosis.2007.11.029>
- Gaj, T., Gersbach, C. A., & Barbas, C. F., 3rd. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*, 31(7), 397-405. <https://doi.org/10.1016/j.tibtech.2013.04.004>
- Gao, D., & Liang, F. S. (2018). Chemical Inducible dCas9-Guided Editing of H3K27 Acetylation in Mammalian Cells. *Methods in Molecular Biology*, 1767, 429-445. [https://doi.org/10.1007/978-1-4939-7774-1\\_24](https://doi.org/10.1007/978-1-4939-7774-1_24)
- Garcia-Bløj, B., Moses, C., Sgro, A., Plani-Lam, J., Arooj, M., Duffy, C., Thiruvengadam, S., Sorolla, A., Rashwan, R., Mancera, R. L., Leisewitz, A., Swift-Scanlan, T., Corvalan, A. H., & Blancafort, P. (2016). Waking up dormant tumor suppressor genes with zinc fingers, TALEs and the CRISPR/dCas9 system. *Oncotarget*, 7(37), 60535-60554. <https://doi.org/10.18632/oncotarget.11142>
- Gjaltema, R. A. F., & Rots, M. G. (2020). Advances of epigenetic editing. *Current Opinion in Chemical Biology*, 57, 75-81. <https://doi.org/10.1016/j.cbpa.2020.04.020>
- Grant, S., Easley, C., & Kirkpatrick, P. (2007). Vorinostat. *Nature Reviews: Drug Discovery*, 6(1), 21-22. <https://doi.org/10.1038/nrd2227>
- Gu, M., Ren, B., Fang, Y., Ren, J., Liu, X., Wang, X., Zhou, F., Xiao, R., Luo, X., You, L., & Zhao, Y. (2024). Epigenetic regulation in cancer. *MedComm (2020)*, 5(2), e495. <https://doi.org/10.1002/mco2.495>
- Ho, T. C. S., Chan, A. H. Y., & Ganesan, A. (2020). Thirty Years of HDAC Inhibitors: 2020 Insight and Hindsight. *Journal of Medicinal Chemistry*, 63(21), 12460-12484. <https://doi.org/10.1021/acs.jmedchem.0c00830>
- Hsu, M. N., Liao, H. T., Truong, V. A., Huang, K. L., Yu, F. J., Chen, H. H., Nguyen, T. K. N., Makarevich, P., Parfyonova, Y., & Hu, Y. C. (2019). CRISPR-based Activation of Endogenous Neurotrophic Genes in Adipose Stem Cell Sheets to Stimulate Peripheral Nerve Regeneration. *Theranostics*, 9(21), 6099-6111. <https://doi.org/10.7150/thno.36790>
- Hsu, J., Lei, Y., Wong, W. K., Liu, S., Lee, K. C., He, X., You, W., Zhou, R., Guo, J. T., Chen, X., Peng, X., Sun, H., Huang, H., Zhao, H., & Feng, B. (2014). Direct activation of human and mouse Oct4 genes using engineered TALE and Cas9 transcription factors. *Nucleic Acids Research*, 42(7), 4375-4390. <https://doi.org/10.1093/nar/gku109>
- Hurtado, C., Acevedo Saenz, L. Y., Vasquez Trespalacios, E. M., Urrego, R., Jenks, S., Sanz, I., & Vasquez, G. (2020). DNA methylation changes on immune cells in Systemic Lupus Erythematosus. *Autoimmunity*, 53(3), 114-121. <https://doi.org/10.1080/08916934.2020.1722108>
- Ichikawa, D. M., Abdin, O., Alerasool, N., Kogenaru, M., Mueller, A. L., Wen, H., Giganti, D. O., Goldberg, G. W., Adams, S., Spencer, J. M., Razavi, R., Nim, S., Zheng, H., Gionco, C., Clark, F. T., Strokach, A., Hughes, T. R., Lionnet, T., Taipale, M.,...Noyes, M. B. (2023). A universal deep-learning model for zinc finger design enables transcription factor reprogramming. *Nature Biotechnology*, 41(8), 1117-1129. <https://doi.org/10.1038/s41587-022-01624-4>
- Jakovcevski, M., & Akbarian, S. (2012). Epigenetic mechanisms in neurological disease. *Nature Medicine*, 18(8), 1194-1204. <https://doi.org/10.1038/nm.2828>
- Jallow, M. B., Huang, K., & Qiu, M. (2025). Versatility of LNPs across different administration routes for targeted RNA delivery. *J Mater Chem B*, 13(26), 7637-7652. <https://doi.org/10.1039/d5tb00575b>
- Jeffries, M. A. (2018). Epigenetic editing: How cutting-edge targeted epigenetic modification might provide novel avenues for autoimmune disease therapy. *Clinical Immunology*, 196, 49-58. <https://doi.org/10.1016/j.clim.2018.02.001>
- Jin, Z., & Liu, Y. (2018). DNA methylation in human diseases. *Genes & Diseases*, 5(1), 1-8. <https://doi.org/10.1016/j.gendis.2018.01.002>
- Kannan, S., Altae-Tran, H., Zhu, S., Xu, P., Streibinger, D., Oshiro, R., Faure, G., Moeller, L., Pham, J., Mears, K. S., Ni, H. M., Macrae, R. K., & Zhang, F. (2025). Evolution-guided protein design of IscB for persistent epigenome editing in vivo. *Nature Biotechnology*. <https://doi.org/10.1038/s41587-025-02655-3>
- Karbassi, E., Padgett, R., Bertero, A., Reinecke, H., Klaiman, J. M., Yang, X., Hauschka, S. D., & Murry, C. E. (2024). Targeted CRISPR activation is functional in engineered human pluripotent stem cells but undergoes silencing after differentiation into cardiomyocytes and endothelium. *Cellular and Molecular Life Sciences*, 81(1), 95. <https://doi.org/10.1007/s00018-023-05101-2>
- Katayama, S., Watanabe, M., Kato, Y., Nomura, W., & Yamamoto, T. (2024). Engineering of Zinc Finger Nucleases Through Structural Modeling Improves Genome Editing Efficiency in Cells. *Advanced Sciences*, 11(23), e2310255. <https://doi.org/10.1002/advs.202310255>
- Kazimierczyk, M., & Wrzesinski, J. (2021). Long Non-Coding RNA Epigenetics. *International Journal of Molecular Sciences*, 22(11). <https://doi.org/10.3390/ijms22116166>
- Kearns, N. A., Pham, H., Tabak, B., Genga, R. M., Silverstein, N. J., Garber, M., & Maehr, R. (2015). Functional annotation of native enhancers with a Cas9-histone demethylase fusion. *Nature Methods*, 12(5), 401-403. <https://doi.org/10.1038/nmeth.3325>
- Khan, S. H. (2019). Genome-Editing Technologies: Concept, Pros, and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application. *Molecular Therapy Nucleic Acids*, 16, 326-334. <https://doi.org/10.1016/j.omtn.2019.02.027>
- Kikuchi, M., Morita, S., Wakamori, M., Sato, S., Uchikubo-Kamo, T., Suzuki, T., Dohmae, N., Shirouzu, M., & Umehara, T. (2023). Epigenetic mechanisms to propagate histone acetylation by p300/CBP. *Nature Communication*, 14(1), 4103. <https://doi.org/10.1038/s41467-023-39735-4>
- Kim, J. M., Kim, K., Schmidt, T., Punj, V., Tucker, H., Rice, J. C., Ulmer, T. S., & An, W. (2015). Cooperation between SMYD3 and PC4 drives a distinct transcriptional program in cancer cells. *Nucleic Acids Research*, 43(18), 8868-8883. <https://doi.org/10.1093/nar/gkv874>
- Kloet, S. L., Whiting, J. L., Gafken, P., Ranish, J., & Wang, E. H. (2012). Phosphorylation-dependent regulation of cyclin D1 and cyclin A gene transcription by TFIIID subunits TAF1 and TAF7. *Molecular and Cellular Biology*, 32(16), 3358-3369. <https://doi.org/10.1128/MCB.00416-12>
- Kwon, D. Y., Zhao, Y. T., Lamonica, J. M., & Zhou, Z. (2017). Locus-specific histone deacetylation using a synthetic CRISPR-Cas9-based HDAC. *Nature Communication*, 8, 15315. <https://doi.org/10.1038/ncomms15315>

- Lakshminarasimhan, R., & Liang, G. (2016). The Role of DNA Methylation in Cancer. *Advances in Experimental Medicine and Biology*, 945, 151-172. [https://doi.org/10.1007/978-3-319-43624-1\\_7](https://doi.org/10.1007/978-3-319-43624-1_7)
- Lauffer, B. I., & Singh, S. M. (2015). Strategies for precision modulation of gene expression by epigenome editing: an overview. *Epigenetics Chromatin*, 8(1), 34. <https://doi.org/10.1186/s13072-015-0023-7>
- Lee, H. B., Sundberg, B. N., Sigafoos, A. N., & Clark, K. J. (2016). Genome Engineering with TALE and CRISPR Systems in Neuroscience [Review]. *Frontiers in Genetics*, 7, 47. <https://doi.org/10.3389/fgene.2016.00047>
- Lensch, S., Herschl, M. H., Ludwig, C. H., Sinha, J., Hinks, M. M., Mukund, A., Fujimori, T., & Bintu, L. (2022). Dynamic spreading of chromatin-mediated gene silencing and reactivation between neighboring genes in single cells. *Elife*, 11. <https://doi.org/10.7554/eLife.75115>
- Li, H., Yang, Y., Hong, W., Huang, M., Wu, M., & Zhao, X. (2020a). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduction and Targeted Therapy*, 5(1), 1. <https://doi.org/10.1038/s41392-019-0089-y>
- Li, K., Liu, Y., Cao, H., Zhang, Y., Gu, Z., Liu, X., Yu, A., Kaphle, P., Dickerson, K. E., Ni, M., & Xu, J. (2020b). Interrogation of enhancer function by enhancer-targeting CRISPR epigenetic editing. *Nature Communication*, 11(1), 485. <https://doi.org/10.1038/s41467-020-14362-5>
- Li, R., Xia, X., Wang, X., Sun, X., Dai, Z., Huo, D., Zheng, H., Xiong, H., He, A., & Wu, X. (2020c). Generation and validation of versatile inducible CRISPRi embryonic stem cell and mouse model. *PLoS Biology*, 18(11), e3000749. <https://doi.org/10.1371/journal.pbio.3000749>
- Li, X., Li, C., & Sun, G. (2016). Histone Acetylation and Its Modifiers in the Pathogenesis of Diabetic Nephropathy. *J Diabetes Res*, 2016, 4065382. <https://doi.org/10.1155/2016/4065382>
- Li, Y. (2021). Modern epigenetics methods in biological research. *Methods*, 187, 104-113. <https://doi.org/10.1016/j.ymeth.2020.06.022>
- Liesenfelder, S., Elsafi Mabrouk, M. H., Iliescu, J., Baranda, M. V., Mizi, A., Perez-Correa, J. F., Wessiepe, M., Papantonis, A., & Wagner, W. (2025). Epigenetic editing at individual age-associated CpGs affects the genome-wide epigenetic aging landscape. *Nature Aging*, 5(6), 997-1009. <https://doi.org/10.1038/s43587-025-00841-1>
- Liu, P., Chen, M., Liu, Y., Qi, L. S., & Ding, S. (2018). CRISPR-Based Chromatin Remodeling of the Endogenous Oct4 or Sox2 Locus Enables Reprogramming to Pluripotency. *Cell Stem Cell*, 22(2), 252-261 e254. <https://doi.org/10.1016/j.stem.2017.12.001>
- Liu, R., Wu, J., Guo, H., Yao, W., Li, S., Lu, Y., Jia, Y., Liang, X., Tang, J., & Zhang, H. (2023). Post-translational modifications of histones: Mechanisms, biological functions, and therapeutic targets. *MedComm*, 4(3), e292. <https://doi.org/10.1002/mco2.292>
- Liu, W., Zhang, S., & Wang, J. (2022). IFN-gamma, should not be ignored in SLE. *Frontiers in Immunology*, 13, 954706. <https://doi.org/10.3389/fimmu.2022.954706>
- Loda, A., & Heard, E. (2019). Xist RNA in action: Past, present, and future. *Plos Genetics*, 15(9), e1008333. <https://doi.org/10.1371/journal.pgen.1008333>
- Lodde, V., Murgia, G., Simula, E. R., Steri, M., Floris, M., & Idda, M. L. (2020). Long Noncoding RNAs and Circular RNAs in Autoimmune Diseases. *Biomolecules*, 10(7). <https://doi.org/10.3390/biom10071044>
- Loscalzo, J., & Handy, D. E. (2014). Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). *Pulmonary Circulation*, 4(2), 169-174. <https://doi.org/10.1086/675979>
- Lu, Y., Chan, Y. T., Tan, H. Y., Li, S., Wang, N., & Feng, Y. (2020). Epigenetic regulation in human cancer: the potential role of epi-drug in cancer therapy. *Molecular Cancer*, 19(1), 79. <https://doi.org/10.1186/s12943-020-01197-3>
- Lunnon, K., Smith, R., Hannon, E., De Jager, P. L., Srivastava, G., Volta, M., Troakes, C., Al-Sarraj, S., Burrage, J., Macdonald, R., Condliffe, D., Harries, L. W., Katsel, P., Haroutunian, V., Kaminsky, Z., Joachim, C., Powell, J., Lovestone, S., Bennett, D. A.,...Mill, J. (2014). Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nature Neuroscience*, 17(9), 1164-1170. <https://doi.org/10.1038/nn.3782>
- Ma, W., Wang, W., Zhao, L., Fan, J., Liu, L., Huang, L., Peng, B., Wang, J., Xu, B., Liu, H., Wu, D., & Zheng, Z. (2025). Reprogramming to restore youthful epigenetics of senescent nucleus pulposus cells for mitigating intervertebral disc degeneration and alleviating low back pain. *Bone Research*, 13(1), 35. <https://doi.org/10.1038/s41413-025-00416-1>
- Mabe, N. W., Perry, J. A., Malone, C. F., & Stegmaier, K. (2024). Pharmacological targeting of the cancer epigenome. *Nature Cancer*, 5(6), 844-865. <https://doi.org/10.1038/s43018-024-00777-2>
- Maeder, M. L., Angstman, J. F., Richardson, M. E., Linder, S. J., Cascio, V. M., Tsai, S. Q., Ho, Q. H., Sander, J. D., Reyon, D., Bernstein, B. E., Costello, J. F., Wilkinson, M. F., & Joung, J. K. (2013a). Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nature Biotechnology*, 31(12), 1137-1142. <https://doi.org/10.1038/nbt.2726>
- Maeder, M. L., Angstman, J. F., Richardson, M. E., Linder, S. J., Cascio, V. M., Tsai, S. Q., Ho, Q. H., Sander, J. D., Reyon, D., Bernstein, B. E., Costello, J. F., Wilkinson, M. F., & Joung, J. K. (2013b). Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nature biotechnology*, 31(12), 1137. <https://doi.org/10.1038/nbt.2726>
- Martinez-Iglesias, O., Naidoo, V., Carrera, I., Corzo, L., & Cacabelos, R. (2023). Natural Bioactive Products as Epigenetic Modulators for Treating Neurodegenerative Disorders. *Pharmaceuticals (Basel, Switzerland)*, 16(2). <https://doi.org/10.3390/ph16020216>
- Martinez-Zamudio, R., & Ha, H. C. (2012). Histone ADP-ribosylation facilitates gene transcription by directly remodeling nucleosomes. *Molecular and Cellular Biology*, 32(13), 2490-2502. <https://doi.org/10.1128/MCB.06667-11>
- Matatiele, P., Tikly, M., Tarr, G., & Gulumian, M. (2015). DNA methylation similarities in genes of black South Africans with systemic lupus erythematosus and systemic sclerosis. *Journal of Biomedical Science*, 22(1), 34. <https://doi.org/10.1186/s12929-015-0142-2>
- McGurk, L., Rifai, O. M., & Bonini, N. M. (2019). Poly(ADP-Ribosylation) in Age-Related Neurological Disease. *Trends in Genetics*, 35(8), 601-613. <https://doi.org/10.1016/j.tig.2019.05.004>
- McPherson, R., & Tybjaerg-Hansen, A. (2016). Genetics of Coronary Artery Disease. *Circulation Research*, 118(4),

- 564-578.  
<https://doi.org/10.1161/CIRCRESAHA.115.306566>
- Mendenhall, E. M., Williamson, K. E., Reyon, D., Zou, J. Y., Ram, O., Joung, J. K., & Bernstein, B. E. (2013). Locus-specific editing of histone modifications at endogenous enhancers. *Nature Biotechnology*, 31(12), 1133-1136.  
<https://doi.org/10.1038/nbt.2701>
- Meriesh, H. A., Lerner, A. M., Chandrasekharan, M. B., & Strahl, B. D. (2020). The histone H4 basic patch regulates SAGA-mediated H2B deubiquitination and histone acetylation. *Journal of Biological Chemistry*, 295(19), 6561-6569.  
<https://doi.org/10.1074/jbc.RA120.013196>
- Miao, F., Chen, Z., Zhang, L., Liu, Z., Wu, X., Yuan, Y. C., & Natarajan, R. (2012). Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. *Journal of Biological Chemistry*, 287(20), 16335-16345.  
<https://doi.org/10.1074/jbc.M111.330373>
- Migliore, L., & Coppede, F. (2009). Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. *Mutation Research*, 667 (1-2), 82-97.  
<https://doi.org/10.1016/j.mrfmmm.2008.10.011>
- Miller, S., Tsou, P. S., Coit, P., Gensterblum-Miller, E., Renauer, P., Rohraff, D. M., Kilian, N. C., Schonfeld, M., & Sawalha, A. H. (2019). Hypomethylation of STAT1 and HLA-DRB1 is associated with type-I interferon-dependent HLA-DRB1 expression in lupus CD8+ T cells. *Annals of the Rheumatic Diseases*, 78(4), 519-528.  
<https://doi.org/10.1136/annrheumdis-2018-214323>
- Mirahmadi, M., Kahani, S. M., Sharifi-Zarchi, A., Firouzabadi, S. G., Behjati, F., & Garshasbi, M. (2025). Genetic Heterogeneity of Autism Spectrum Disorder: Identification of Five Novel Mutations (RIMS2, FOXG1, AUTS2, ZCCHC17, and SPTBN5) in Iranian Families via Whole-Exome and Whole-Genome Sequencing. *Biochemical Genetics*.  
<https://doi.org/10.1007/s10528-025-11226-9>
- Mock, U., Riecken, K., Berdien, B., Qasim, W., Chan, E., Cathomen, T., & Fehse, B. (2014). Novel lentiviral vectors with mutated reverse transcriptase for mRNA delivery of TALE nucleases. *Scientific Reports*, 4, 6409.  
<https://doi.org/10.1038/srep06409>
- Morgan, M. T., & Wolberger, C. (2017). Recognition of ubiquitinated nucleosomes. *Current Opinion in Structural Biology*, 42, 75-82.  
<https://doi.org/10.1016/j.sbi.2016.11.016>
- Morlando, M., & Fatica, A. (2018). Alteration of Epigenetic Regulation by Long Noncoding RNAs in Cancer. *International Journal of Molecular Sciences*, 19(2).  
<https://doi.org/10.3390/ijms19020570>
- Nakamura, M., Gao, Y., Dominguez, A. A., & Qi, L. S. (2021). CRISPR technologies for precise epigenome editing. *Nature Cell Biology*, 23(1), 11-22.  
<https://doi.org/10.1038/s41556-020-00620-7>
- Noori-Zadeh, A., Mesbah-Namin, S. A., & Saboor-Yaraghi, A. A. (2017). Epigenetic and gene expression alterations of FOXP3 in the T cells of EAE mouse model of multiple sclerosis. *Journal of the Neurological Sciences*, 375, 203-208. <https://doi.org/10.1016/j.jns.2017.01.060>
- Nunez, J. K., Chen, J., Pommier, G. C., Cogan, J. Z., Replogle, J. M., Adriaens, C., Ramadoss, G. N., Shi, Q., Hung, K. L., Samelson, A. J., Pogson, A. N., Kim, J. Y. S., Chung, A., Leonetti, M. D., Chang, H. Y., Kampmann, M., Bernstein, B. E., Hovestadt, V., Gilbert, L. A., & Weissman, J. S. (2021). Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell*, 184(9), 2503-2519 e2517.  
<https://doi.org/10.1016/j.cell.2021.03.025>
- O'Geen, H., Ren, C., Nicolet, C. M., Perez, A. A., Halmaj, J., Le, V. M., Mackay, J. P., Farnham, P. J., & Segal, D. J. (2017). dCas9-based epigenome editing suggests acquisition of histone methylation is not sufficient for target gene repression. *Nucleic Acids Research*, 45(17), 9901-9916.  
<https://doi.org/10.1093/nar/gkx578>
- Ordovas, J. M., & Smith, C. E. (2010). Epigenetics and cardiovascular disease. *Nature Reviews: Cardiology*, 7(9), 510-519. <https://doi.org/10.1038/nrcardio.2010.104>
- Palazzo, L., Mikolcovic, P., Mikoc, A., & Ahel, I. (2019). ADP-ribosylation signalling and human disease. *Open Biology*, 9(4), 190041. <https://doi.org/10.1098/rsob.190041>
- Pang, K., Wang, W., Qin, J. X., Shi, Z. D., Hao, L., Ma, Y. Y., Xu, H., Wu, Z. X., Pan, D., Chen, Z. S., & Han, C. H. (2022). Role of protein phosphorylation in cell signaling, disease, and the intervention therapy. *MedComm (2020)*, 3(4), e175.  
<https://doi.org/10.1002/mco2.175>
- Pedre, X., Mastronardi, F., Bruck, W., Lopez-Rodas, G., Kuhlmann, T., & Casaccia, P. (2011). Changed histone acetylation patterns in normal-appearing white matter and early multiple sclerosis lesions. *Journal of Neuroscience*, 31(9), 3435-3445.  
<https://doi.org/10.1523/JNEUROSCI.4507-10.2011>
- Polcarpi, C., Munafo, M., Tsagkris, S., Carlini, V., & Hackett, J. A. (2024). Systematic epigenome editing captures the context-dependent instructive function of chromatin modifications. *Nature Genetics*, 56(6), 1168-1180.  
<https://doi.org/10.1038/s41588-024-01706-w>
- Poole, R. M. (2014). Belinostat: first global approval. *Drugs*, 74(13), 1543-1554.  
<https://doi.org/10.1007/s40265-014-0275-8>
- Porcheron, C., Le Devehat, M., Roubtsova, A., Bayat, H., Evagelidis, A., Jafarzadeh, L., Sachan, V., Labrecque, N., Fonta Holder, A., Susan-Resiga, D., Essalmani, R., Boudreau, G., Prat, A., Cussedu, R., Cote, J. F., Khatib, A. M., Delisle, J. S., & Seidah, N. G. (2025). Blockade of colon cancer metastasis via single and double silencing of PCSK7/PCSK9: enhanced T cells cytotoxicity in mouse and human. *Journal of Immunotherapy of Cancer*, 13(6).  
<https://doi.org/10.1136/jitc-2024-011364>
- Qin, S., Xie, B., Wang, Q., Yang, R., Sun, J., Hu, C., Liu, S., Tao, Y., & Xiao, D. (2024). New insights into immune cells in cancer immunotherapy: from epigenetic modification, metabolic modulation to cell communication. *MedComm (2020)*, 5(6), e551.  
<https://doi.org/10.1002/mco2.551>
- Qin, W., Scicluna, B. P., & van der Poll, T. (2021). The Role of Host Cell DNA Methylation in the Immune Response to Bacterial Infection [Review]. *Frontiers in Immunology*, 12, 696280.  
<https://doi.org/10.3389/fimmu.2021.696280>
- Ramzan, F., Vickers, M. H., & Mithen, R. F. (2021). Epigenetics, microRNA and Metabolic Syndrome: A Comprehensive Review. *International Journal of Molecular Sciences*, 22(9).  
<https://doi.org/10.3390/ijms22095047>
- Rasmi, Y., Shokati, A., Hassan, A., Aziz, S. G., Bastani, S., Jalali, L., Moradi, F., & Alipour, S. (2023). The role of DNA methylation in progression of neurological disorders and neurodegenerative diseases as well as the prospect of using DNA methylation inhibitors as therapeutic agents for such disorders. *IBRO Neuroscience Reports*. 14. 28-

37. <https://doi.org/10.1016/j.ibneur.2022.12.002>
- Richard-Miceli, C., & Criswell, L. A. (2012). Emerging patterns of genetic overlap across autoimmune disorders. *Genome Medicine*, 4(1), 6. <https://doi.org/10.1186/gm305>
- Richardson, B. (2003). DNA methylation and autoimmune disease. *Clinical Immunology*, 109(1), 72-79. [https://doi.org/10.1016/s1521-6616\(03\)00206-7](https://doi.org/10.1016/s1521-6616(03)00206-7)
- Robusti, G., Vai, A., Bonaldi, T., & Nuberini, R. (2022). Investigating pathological epigenetic aberrations by epiproteomics. *Clinical Epigenetics*, 14(1), 145. <https://doi.org/10.1186/s13148-022-01371-y>
- Rosenblum, M. D., Remedios, K. A., & Abbas, A. K. (2015). Mechanisms of human autoimmunity. *Journal of Clinical Investigation*, 125(6), 2228-2233. <https://doi.org/10.1172/JCI78088>
- Roth, G. V., Gengaro, I. R., & Qi, L. S. (2024). Precision epigenetic editing: Technological advances, enduring challenges, and therapeutic applications. *Cell Chemical Biology*. <https://doi.org/10.1016/j.chembiol.2024.07.007>
- Ryu, H. Y., & Hochstrasser, M. (2021). Histone sumoylation and chromatin dynamics. *Nucleic Acids Research*, 49(11), 6043-6052. <https://doi.org/10.1093/nar/gkab280>
- Ryu, H. Y., Zhao, D., Li, J., Su, D., & Hochstrasser, M. (2020). Histone sumoylation promotes Set3 histone-deacetylase complex-mediated transcriptional regulation. *Nucleic Acids Research*, 48(21), 12151-12168. <https://doi.org/10.1093/nar/gkaa1093>
- Saito, M., Xu, P., Faure, G., Maguire, S., Kannan, S., Altae-Tran, H., Vo, S., Desimone, A., Macrae, R. K., & Zhang, F. (2023). Fanzor is a eukaryotic programmable RNA-guided endonuclease. *Nature*, 620(7974), 660-668. <https://doi.org/10.1038/s41586-023-06356-2>
- Saito, Y., Saito, H., Liang, G., & Friedman, J. M. (2014). Epigenetic alterations and microRNA misexpression in cancer and autoimmune diseases: a critical review. *Clinical Reviews in Allergy & Immunology*, 47(2), 128-135. <https://doi.org/10.1007/s12016-013-8401-z>
- Salesi, M., Dehabadi, M. H., Salehi, R., Salehi, A., & Pakzad, B. (2022). Differential methylation of IFI44L gene promoter in Iranian patients with systemic lupus erythematosus and rheumatoid arthritis. *Molecular Biology Reports*, 49(4), 3065-3072. <https://doi.org/10.1007/s11033-022-07134-5>
- Salmaninejad, A., Jafari Abarghan, Y., Bozorg Qomi, S., Bayat, H., Yousefi, M., Azhdari, S., Talebi, S., & Mojarad, M. (2021). Common therapeutic advances for Duchenne muscular dystrophy (DMD). *International Journal of Neuroscience*, 131(4), 370-389. <https://doi.org/10.1080/00207454.2020.1740218>
- Salmaninejad, A., Valilou, S. F., Bayat, H., Ebadi, N., Daraei, A., Yousefi, M., Nesaee, A., & Mojarad, M. (2018). Duchenne muscular dystrophy: an updated review of common available therapies. *International Journal of Neuroscience*, 128(9), 854-864. <https://doi.org/10.1080/00207454.2018.1430694>
- Salvatori, B., Biscarini, S., & Morlando, M. (2020). Non-coding RNAs in Nervous System Development and Disease [Review]. *Front Cell Dev Biol*, 8, 273. <https://doi.org/10.3389/fcell.2020.00273>
- Samie, M., Parman, T., Jalan, M., Lee, J., Dunn, P., Eshleman, J., Vidales, D. B., Holter, J., Jones, B., Pan, Y., Falaleeva, M., Hinkley, S., Goodwin, A., Chen, T., Bhardwaj, S., Ward, A., Trias, M., Chikere, A., Som, M., Pooler, A. (2024). Potent and selective repression of SCN9A by engineered zinc finger repressors for the treatment of neuropathic pain. *BioRxiv*. <https://doi.org/10.1101/2024.09.06.609976>
- San-Miguel, J. F., Hungria, V. T., Yoon, S. S., Beksac, M., Dimopoulos, M. A., Elghandour, A., Jedrzejczak, W. W., Gunther, A., Nakorn, T. N., Siritanaratkul, N., Corradini, P., Chuncharunee, S., Lee, J. J., Schlossman, R. L., Shelekhova, T., Yong, K., Tan, D., Numbenjapon, T., Cavenagh, J. D., Richardson, P. G. (2014). Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. *Lancet Oncology*, 15(11), 1195-1206. [https://doi.org/10.1016/S1470-2045\(14\)70440-1](https://doi.org/10.1016/S1470-2045(14)70440-1)
- Sanjana, N. E., Cong, L., Zhou, Y., Cunniff, M. M., Feng, G., & Zhang, F. (2012). A transcription activator-like effector toolbox for genome engineering. *Nature Protocols*, 7(1), 171-192. <https://doi.org/10.1038/nprot.2011.431>
- Saunderson, E. A., Encabo, H. H., Devis, J., Rouault-Pierre, K., Piganeau, M., Bell, C. G., Gribben, J. G., Bonnet, D., & Ficiz, G. (2023). CRISPR/dCas9 DNA methylation editing is heritable during human hematopoiesis and shapes immune progeny. *Proceedings of the National Academy of Sciences of the United States of America*, 120(34), e2300224120. <https://doi.org/10.1073/pnas.2300224120>
- Seem, K., Kaur, S., Kumar, S., & Mohapatra, T. (2024). Epigenome editing for targeted DNA (de)methylation: a new perspective in modulating gene expression. *Critical Reviews in Biochemistry and Molecular Biology*, 59(1-2), 69-98. <https://doi.org/10.1080/10409238.2024.2320659>
- Shah, R. R. (2019). Safety and Tolerability of Histone Deacetylase (HDAC) Inhibitors in Oncology. *Drug Safety*, 42(2), 235-245. <https://doi.org/10.1007/s40264-018-0773-9>
- Shams, F., Bayat, H., Mohammadian, O., Mahboudi, S., Vahidnezhad, H., Soosanabadi, M., & Rahimpour, A. (2022). Advance trends in targeting homology-directed repair for accurate gene editing: An inclusive review of small molecules and modified CRISPR-Cas9 systems. *Bioimpacts*, 12(4), 371-391. <https://doi.org/10.34172/bi.2022.23871>
- Sharma, V. K., Mehta, V., & Singh, T. G. (2020). Alzheimer's Disorder: Epigenetic Connection and Associated Risk Factors. *Current Neuropharmacology*, 18(8), 740-753. <https://doi.org/10.2174/1570159X18666200128125641>
- Shi, L., Li, S., Zhu, R., Lu, C., Xu, X., Li, C., Huang, X., Zhao, X., Mao, F., & Li, K. (2025). CRISPRepi: a multi-omic atlas for CRISPR-based epigenome editing. *Nucleic Acids Research*, 53(D1), D901-D913. <https://doi.org/10.1093/nar/gkae1039>
- Shrivastava, S., Ray, R. M., Holguin, L., Echavarría, L., Grepo, N., Scott, T. A., Burnett, J., & Morris, K. V. (2021). Exosome-mediated stable epigenetic repression of HIV-1. *Nature Communication*, 12(1), 5541. <https://doi.org/10.1038/s41467-021-25839-2>
- Shukla, S., & Tekwani, B. L. (2020). Histone Deacetylases Inhibitors in Neurodegenerative Diseases, Neuroprotection and Neuronal Differentiation. *Frontiers in Pharmacology*, 11, 537. <https://doi.org/10.3389/fphar.2020.00537>
- Siddiqi, K. Z., Wilhelm, T. R., Ulf-Moller, C. J., & Jacobsen, S. (2021). Cluster of highly expressed interferon-stimulated

- genes associate more with African ancestry than disease activity in patients with systemic lupus erythematosus. A systematic review of cross-sectional studies. *Translational Research: The Journal of Laboratory and Clinical Medicine*, 238, 63-75. <https://doi.org/10.1016/j.trsl.2021.07.006>
- Singh, J., & Santosh, P. (2025). Molecular Insights into Neurological Regression with a Focus on Rett Syndrome- A Narrative Review. *International Journal of Molecular Sciences*, 26(11). <https://doi.org/10.3390/ijms26115361>
- Skeens, E., Sinha, S., Ahsan, M., D'Ordine, A. M., Jogl, G., Palermo, G., & Lisi, G. P. (2024). High-fidelity, hyper-accurate, and evolved mutants rewire atomic-level communication in CRISPR-Cas9. *Science Advances*, 10(10), eadl1045. <https://doi.org/10.1126/sciadv.adl1045>
- Snowden, A. W., Gregory, P. D., Case, C. C., & Pabo, C. O. (2002). Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. *Current Biology*, 12(24), 2159-2166. [https://doi.org/10.1016/s0960-9822\(02\)01391-x](https://doi.org/10.1016/s0960-9822(02)01391-x)
- Song, S., De, S., Nelson, V., Chopra, S., LaPan, M., Kampta, K., Sun, S., He, M., Thompson, C. D., Li, D., Shih, T., Tan, N., Al-Abed, Y., Capitle, E., Aranow, C., Mackay, M., Clapp, W. L., & Barnes, B. J. (2020). Inhibition of IRF5 hyperactivation protects from lupus onset and severity. *Journal of Clinical Investigation*, 130(12), 6700-6717. <https://doi.org/10.1172/JCI120288>
- Sum, H., & Brewer, A. C. (2023). Epigenetic modifications as therapeutic targets in atherosclerosis: a focus on DNA methylation and non-coding RNAs. *Frontiers in Cardiovascular Medicine*, 10, 1183181. <https://doi.org/10.3389/fcvm.2023.1183181>
- Syding, L. A., Nickl, P., Kasperek, P., & Sedlacek, R. (2020). CRISPR/Cas9 Epigenome Editing Potential for Rare Imprinting Diseases: A Review. *Cells*, 9(4). <https://doi.org/10.3390/cells9040993>
- Tadic, V., Josipovic, G., Zoldos, V., & Vojta, A. (2019). CRISPR/Cas9-based epigenome editing: An overview of dCas9-based tools with special emphasis on off-target activity. *Methods*, 164-165, 109-119. <https://doi.org/10.1016/j.ymeth.2019.05.003>
- Tang, C., Li, Y., Lin, X., Ye, J., Li, W., He, Z., Li, F., & Cai, X. (2014). Hypomethylation of interleukin 6 correlates with renal involvement in systemic lupus erythematosus. *Central European Journal of Immunology*, 39(2), 203-208. <https://doi.org/10.5114/cej.2014.43724>
- Tang, H., Wang, D., & Shu, Y. (2022). Structural insights into Cas9 mismatch: promising for development of high-fidelity Cas9 variants. *Signal Transduction and Targeted Therapy*, 7(1), 271. <https://doi.org/10.1038/s41392-022-01139-z>
- Tenchov, R., Sasso, J. M., Wang, X., Liaw, W. S., Chen, C. A., & Zhou, Q. A. (2022). Exosomes horizontal line Nature's Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics. *ACS Nano*, 16(11), 17802-17846. <https://doi.org/10.1021/acsnano.2c08774>
- Tremblay, F., Xiong, Q., Shah, S. S., Ko, C. W., Kelly, K., Morrison, M. S., Giancarlo, C., Ramirez, R. N., Hildebrand, E. M., Voytek, S. B., El Sebae, G. K., Wright, S. H., Lofgren, L., Clarkson, S., Waters, C., Linder, S. J., Liu, S., Eom, T., Parikh, S., Jaffe, A. B. (2025). A potent epigenetic editor targeting human PCSK9 for durable reduction of low-density lipoprotein cholesterol levels. *Nature Medicine*, 31(4), 1329-1338. <https://doi.org/10.1038/s41591-025-03508-x>
- Udali, S., Guarini, P., Moruzzi, S., Choi, S. W., & Friso, S. (2013). Cardiovascular epigenetics: from DNA methylation to microRNAs. *Molecular Aspects of Medicine*, 34(4), 883-901. <https://doi.org/10.1016/j.mam.2012.08.001>
- Ueda, J., Yamazaki, T., & Funakoshi, H. (2023). Toward the Development of Epigenome Editing-Based Therapeutics: Potentials and Challenges. *International Journal of Molecular Sciences*, 24(5). <https://doi.org/10.3390/ijms24054778>
- Urnov, F. D., Rebar, E. J., Holmes, M. C., Zhang, H. S., & Gregory, P. D. (2010). Genome editing with engineered zinc finger nucleases. *Nature Reviews: Genetics*, 11(9), 636-646. <https://doi.org/10.1038/nrg2842>
- van Esch, A. P., Prudence, S. M. M., Contesini, F. J., Gerhartz, B., Royle, K. E., & Mortensen, U. H. (2025). A CRISPR Cas12a/Cpf1 strategy to facilitate robust multiplex gene editing in *Aspergillus Niger*. *Fungal Biology and Biotechnology*, 12(1), 5. <https://doi.org/10.1186/s40694-025-00196-7>
- Vukic, M., & Daxinger, L. (2019). DNA methylation in disease: Immunodeficiency, Centromeric instability, Facial anomalies syndrome. *Essays in Biochemistry*, 63(6), 773-783. <https://doi.org/10.1042/EBC20190035>
- Wardowska, A. (2020). The epigenetic face of lupus: Focus on antigen-presenting cells. *International Immunopharmacology*, 81, 106262. <https://doi.org/10.1016/j.intimp.2020.106262>
- Whittaker, M. N., Testa, L. C., Quigley, A., Jindal, I., Cortez-Alvarado, S. V., Qu, P., Yang, Y., Alameh, M. G., Musunuru, K., & Wang, X. (2023). Epigenome Editing Durability Varies Widely Across Cardiovascular Disease Target Genes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 43(10), 2075-2077. <https://doi.org/10.1161/ATVBAHA.123.319748>
- Woodward, E. A., Wang, E., Wallis, C., Sharma, R., Tie, A. W. J., Murthy, N., & Blancafort, P. (2024). Protocol for Delivery of CRISPR/dCas9 Systems for Epigenetic Editing into Solid Tumors Using Lipid Nanoparticles Encapsulating RNA. *Methods in Molecular Biology*, 2842, 267-287. [https://doi.org/10.1007/978-1-0716-4051-7\\_14](https://doi.org/10.1007/978-1-0716-4051-7_14)
- Wu, X., Zhang, X., Huang, B., Han, J., & Fang, H. (2023a). Advances in biological functions and mechanisms of histone variants in plants. *Frontiers in Genetics*, 14, 1229782. <https://doi.org/10.3389/fgene.2023.1229782>
- Wu, Y. L., Lin, Z. J., Li, C. C., Lin, X., Shan, S. K., Guo, B., Zheng, M. H., Li, F., Yuan, L. Q., & Li, Z. H. (2023b). Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. *Signal Transduction and Targeted Therapy*, 8(1), 98. <https://doi.org/10.1038/s41392-023-01333-7>
- Xiao, L., Xiao, W., & Lin, S. (2022). Potential biomarkers for active renal involvement in systemic lupus erythematosus patients. *Frontiers in Medicine*, 9, 995103. <https://doi.org/10.3389/fmed.2022.995103>
- Xu, Y., Yang, Y., Wang, Z., Sjöstrom, M., Jiang, Y., Tang, Y., Cheng, S., Deng, S., Wang, C., Gonzalez, J., Johnson, N. A., Li, X., Li, X., Metang, L. A., Mukherji, A., Xu, Q., Tirado, C. R., Wainwright, G., Yu, X.,...Mu, P. (2024). ZNF397 Deficiency Triggers TET2-Driven Lineage Plasticity and AR-Targeted Therapy Resistance in Prostate Cancer. *Cancer Discovery*, 14(8), 1496-1521. <https://doi.org/10.1158/2159-8290.CD-23-0539>

- Yang, X., Liu, M., Li, M., Zhang, S., Hiju, H., Sun, J., Mao, Z., Zheng, M., & Feng, B. (2020). Epigenetic modulations of noncoding RNA: a novel dimension of Cancer biology. *Molecular Cancer*, *19*(1), 64. <https://doi.org/10.1186/s12943-020-01159-9>
- Younesian, S., Yousefi, A. M., Momeny, M., Ghaffari, S. H., & Bashash, D. (2022). The DNA Methylation in Neurological Diseases. *Cells*, *11*(21). <https://doi.org/10.3390/cells11213439>
- Zhan, Y., Guo, Y., & Lu, Q. (2016). Aberrant Epigenetic Regulation in the Pathogenesis of Systemic Lupus Erythematosus and Its Implication in Precision Medicine. *Cytogenetic and Genome Research*, *149*(3), 141-155. <https://doi.org/10.1159/000448793>
- Zhang, J., Chen, L., Zhang, J., & Wang, Y. (2019). Drug Inducible CRISPR/Cas Systems. *Computational and Structural Biotechnology Journal*, *17*, 1171-1177. <https://doi.org/10.1016/j.csbj.2019.07.015>
- Zhang, J., Chen, L. M., Zou, Y., Zhang, S., Xiong, F., & Wang, C. Y. (2021). Implication of epigenetic factors in the pathogenesis of type 1 diabetes. *Chinese Medical Journal (Engl.)*, *134*(9), 1031-1042. <https://doi.org/10.1097/CM9.0000000000001450>
- Zhang, R., Yao, T., Fan, M., Jiang, X., Wang, K., Cui, M., Bing, K., & Xia, X. (2025). Precision scalpels for the epigenome: next-gen editing tools in targeted therapies. *Frontiers in Medicine*, *12*, 1613722. <https://doi.org/10.3389/fmed.2025.1613722>
- Zhang, Y., Liu, L., Guo, S., Song, J., Zhu, C., Yue, Z., Wei, W., & Yi, C. (2017). Deciphering TAL effectors for 5-methylcytosine and 5-hydroxymethylcytosine recognition. *Nature Communication*, *8*(1), 901. <https://doi.org/10.1038/s41467-017-00860-6>
- Zhao, J., Wei, K., Chang, C., Xu, L., Jiang, P., Guo, S., Schrodi, S. J., & He, D. (2022). DNA Methylation of T Lymphocytes as a Therapeutic Target: Implications for Rheumatoid Arthritis Etiology. *Frontiers in Immunology*, *13*, 863703. <https://doi.org/10.3389/fimmu.2022.863703>
- Zhao, Q., Ma, Y., Li, Z., Zhang, K., Zheng, M., & Zhang, S. (2020). The Function of SUMOylation and Its Role in the Development of Cancer Cells under Stress Conditions: A Systematic Review. *Stem Cells International*, *2020*, 8835714. <https://doi.org/10.1155/2020/8835714>
- Zhou, H., He, X., Xiong, Y., Gong, Y., Zhang, Y., Li, S., Hu, R., Li, Y., Zhang, X., Zhou, X., Zhu, J., Yang, Y., & Liu, M. (2025). Structural insights into a highly flexible zinc finger module unravel INSM1 function in transcription regulation. *Nature Communication*, *16*(1), 2162. <https://doi.org/10.1038/s41467-025-57478-2>