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Advances in Epigenome Engineering: Mastering Technical Approaches for Better Outcomes

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Abstract

Epigenomic analysis involves large-scale data science, which poses significant challenges in converting data into usable knowledge. To achieve precise control over gene activation and suppression, a comprehensive understanding of the molecular components of epigenetic processes is essential. Only recently have advancements in technology allowed for the thorough exploration of the functional effects of complex epigenetic pathways. This progress involves integrating nuclease-free genome-editing (GE) systems with effector domains. Contemporary epigenome editing (EpGE) systems can be customized to enable accurate modification of epigenetic marks without altering the DNA sequence itself. This review describes current techniques for epigenetic manipulation and their applications in human health and food. The rise of CRISPR-based EpGE technologies promises to revolutionize the regulation of chromatin and epigenetic markers, providing new opportunities for therapeutic and agricultural purposes. Nevertheless, this emerging field still faces significant hurdles.

Keywords: Gene expression, Epigenetics, Genome, Epigenome, Genomics

Introduction

The genome refers to the complete set of genetic material within an organism, including humans. In contrast, epigenetics—literally meaning "above genetics"—explores modifications that affect gene activity without altering the DNA sequence itself. The study of these modifications across the entire genome is known as epigenomics, which focuses on changes to DNA, histone proteins, and the architecture of the nucleus.

Epigenomic research examines how chromatin structure influences genetic regulation. This includes higher-order chromatin folding, DNA wrapping around histones to form nucleosomes, histone tail modifications (such as acetylation, methylation, phosphorylation, and ubiquitination), and DNA methylation (DM). Additionally, non-coding RNAs (ncRNAs) have recently emerged as key players in epigenetic regulation **(Figure 1)**.

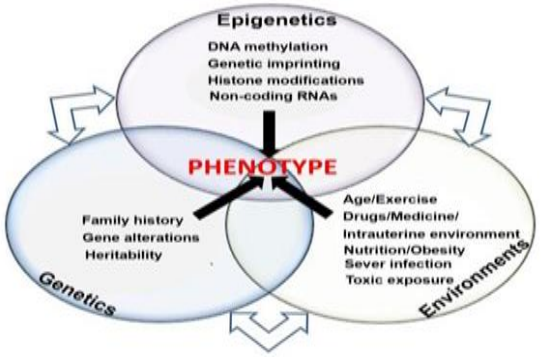

Figure 1. The interplay between genetics, epigenetics, and environmental factors contributes to normal or abnormal phenotypes (Adapted from [1, 2])

These changes, collectively known as histone post-translational modifications, often occur at multiple loci.

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They are accompanied by alterations in CpG-rich DNA regions and changes in ncRNA expression. In the past few years, major strides have been made in uncovering the biological roles of these mechanisms.

Alterations in the epigenome can reshape chromatin organization, directly influencing genome function [3]. Unlike the relatively stable genome, the epigenome is dynamic and can be modulated by environmental stimuli. Epigenetic processes govern gene expression, cell differentiation, and developmental pathways.

Researchers have categorized epigenetic mechanisms into three main types based on their timing and source: direct epigenetics (DE), within indirect epigenetics (WIE), and across indirect epigenetics (AIE) [4]. Direct epigenetics refers to changes occurring within an individual's lifespan. If these alterations are inherited, they become indirect triggers affecting the next generation's development. WIE covers the collective changes during early development, beginning with zygote formation and influenced by its immediate environment. AIE, on the other hand, encompasses inherited epigenetic effects from parents or even grandparents.

These epigenetic changes may or may not be heritable. In some cases, they are passed down through transgenerational epigenetic inheritance, where traits are transferred via epigenetic marks rather than DNA sequence [5]. For example, DNA methyltransferases (DNMTs) are enzymes that maintain DNA methylation after cell division by transferring methyl groups from S-adenosyl methionine to the fifth carbon of cytosine. Demethylation—the reverse process—can happen passively or actively. Enzymes like TET aid in removing these methyl marks [5-7].

DNA and histone methylation typically reduce gene activity, while acetylation and phosphorylation enhance gene expression [8]. Remarkably, DNA methylation memory can persist across generations or even for millions of years, as observed in yeast [5]. This phenomenon, known as cellular memory, allows daughter cells or offspring to inherit gene expression patterns from their predecessors.

Recent advances in biotechnology now make it possible to target and edit specific epigenetic features, leading to major progress in our understanding of epigenetic influence on disease, cellular behavior, and therapeutic approaches. Yet, despite rapid development, many aspects remain poorly understood and require further research.

Interest in epigenetics has grown rapidly since the early 2000s. As shown in **Figure 2**, publications containing the term "epigenetics" increased dramatically between 2000 and 2010, reaching around 1,000 studies. The launch of the human epigenome project further boosted attention. By 2015, the foundational regulatory elements of gene expression across 127 human tissues and cell types were identified, pushing publication numbers beyond 3,000. The exponential growth continued, making it difficult to stay updated. At the same time, biotech companies began producing diagnostic tools and epigenetic drugs [7].

The scale of this progress is underscored by a 2016 report from Grand View Research, which projected the global epigenetics market would hit $16.31 billion by 2022—a testament to the field's growing significance.

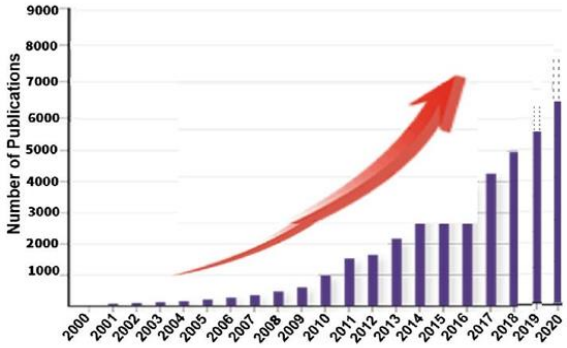

Figure 2. Search results of epigenetic publications per year by each keyword in PubMed and the database of ISI Web of Knowledge.

This review outlines current techniques for epigenetic manipulation and their applications in human health and food.

Materials and Methods

This review is grounded in a detailed analysis of scientific literature available up until July 2023. Data were gathered using two primary databases: PubMed and the ISI Web of Knowledge. A combination of specific search terms was employed to capture relevant content, including "epigenetics," "epigenome editing," "genome editing," "functional genomics," "gene expression," and "CRISPR/Cas9."

Following the removal of duplicate entries and non-English papers, 80 studies were ultimately selected for inclusion. These publications were reviewed to provide an overview of epigenome editing strategies, evaluate

their practical uses, and highlight unresolved challenges within the field. Among the selected works, only two dated back to 2007 and 2010, and were cited to describe the initial concepts of gene regulation and epigenome-editing mechanisms. The remaining articles span from 2011 through 2023, with a notable increase in publications in recent years. More than two-thirds of the included articles were published between 2019 and 2023, indicating a recent surge of research interest and development in epigenome-related studies.

Results and Discussion

Although genome editing and epigenome editing both rely on the ability to bind to specific DNA regions, their underlying goals differ considerably. Genome editing techniques are designed to introduce permanent changes to the DNA sequence itself, often relying on the cell's internal repair systems to finalize those edits. In contrast, epigenome editing focuses on modifying the activity of genes without altering the sequence of nucleotides. It achieves this by changing the chemical environment surrounding the DNA, which influences how genes are expressed.

The principle of epigenome editing is built on two defining properties of epigenetic marks: their ability to be passed on during cell division, and their potential to be reversed in response to environmental factors. Methylation of DNA and modifications of histone proteins are central to this process. These chemical changes act like switches, turning genes on or off depending on cellular context. Enzymes involved in epigenetic regulation can either write new marks or erase existing ones. Effective gene regulation requires the appropriate balance between these two opposing functions.

In practical terms, researchers have developed methods to direct these enzymes to specific locations in the genome by attaching them to a DNA-binding system, often through a fusion protein. This fusion consists of a catalytic or effector domain linked to a sequence-specific binding protein. These systems allow for precise control over where and how a gene is regulated. For example, histone methyltransferases add methyl groups to specific amino acids on histone tails, while demethylases such as the Jumonji family remove them through oxidation-based processes [9-12].

Over the years, various epigenome-editing platforms have been designed to target methylation patterns at specific loci. A major leap in this field came with the adaptation of the CRISPR/Cas9 system—specifically, a catalytically inactivated version known as dCas9. This modified protein retains its ability to bind to DNA but cannot cut it. Researchers have used dCas9 to deliver epigenetic modifiers to chosen sites in the genome, improving specificity and simplifying the process compared to earlier systems like zinc finger proteins or TALEs. While dCas9-based systems provide a more streamlined and customizable method for guiding these effectors, they sometimes produce lower transcriptional activation levels compared to TALE-based systems. Nevertheless, the flexibility of dCas9 has opened new doors for understanding and manipulating gene expression in a more targeted and programmable way **(Figure 3)**.

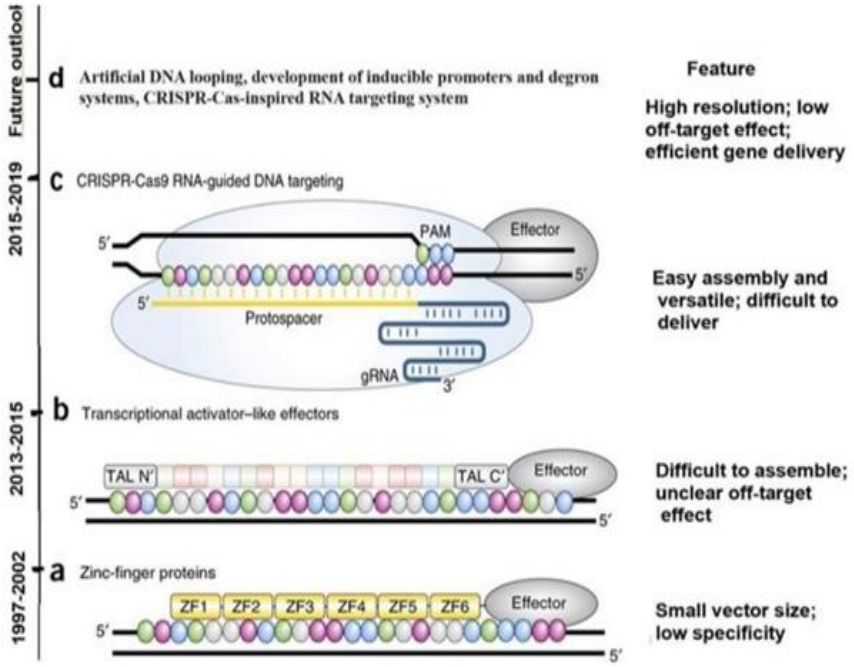

Figure 3. Development and use of epigenome editing technologies; (a) the first tool for targeted DNA methylation was developed in 1997; this in vitro system involved fusing a bacterial methyltransferase (effector domain) with zinc finger proteins (ZFPs) to recognize a specific nine-base DNA sequence; by 2002, ZFPs were also linked to catalytic domains of histone methyltransferases (HMTs) to modify histone H3 at lysine 9 (H3K9) directly within living cells; (b) Later, catalytic domains from DNMT3a, TET hydroxylases, and lysine-specific demethylase 1 (LSD1) were fused with TALE proteins to enable more advanced editing; (c) between 2015 and 2019, the use of catalytically inactive CRISPR-associated protein 9 (dCas9) greatly expanded the range of epigenome editing tools available; and (d) looking ahead, further improvements are expected (Adapted from references 15 and 16).

In 2015, researchers reported that the catalytic domain (CD) of the p300 histone acetyltransferase (HAT) was fused with DNA-targeting systems like CRISPR-dCas9, TALEs, and zinc finger proteins (ZFPs) [13]. This fusion enabled targeted acetylation of histone H3 at lysine 27 (H3K27), along with other histone tail acetylation sites, thereby promoting gene activation **(Table 1)**. Other epigenetic modifiers that perform covalent changes to DNA have also been connected to sequence-specific DNA-binding proteins to regulate gene expression. For instance, TET demethylases have been combined with ZFPs [14], TALEs [11], and CRISPR-dCas9 [15] to demethylate specific gene promoters, which leads to gene activation. These enzymes oxidize 5-methyl-deoxycytosine (m5dC) in a stepwise fashion to form 5-hydroxymethylcytosine, 5-formylcytosine, and finally 5-carboxylcytosine.

Programmable epigenome editing systems

Today, three main types of DNA-binding platforms are used for epigenome editing **(Table 1)**: ZFPs, TALEs [16], and CRISPR-based systems [3, 17]. These systems function by linking customizable DNA recognition domains (ZFPs, TALEs, or dCas9) to catalytic domains of enzymes that modify chromatin [18]. Of these, CRISPR/dCas9 is the most widely used due to its low cost, ease of design, and high versatility [19, 20].

Table 1. DNA-binding modules and their effector domains used in epigenome editing

DNA-targeting module	Effector domain(s)	Function/application
ZFP	M.SssI (bacterial DNMT)	In vitro DNA methylation
	SUV39H1 and G9A catalytic domains	H3K9 methylation at VEGF-A locus in cells

	G9A catalytic domain + NF-kB p65	Histone modifications at FosB locus in mouse brain
	Full/self-association domain of Ldb1	Induces chromatin looping at β-globin or γ-globin loci
TALE	TET1 catalytic domain	In vivo DNA demethylation
	DNMT3a + DNMT3L catalytic domains	In vivo methylation of CDKN2A
	LSD1 (histone demethylase)	H3K4 demethylation at enhancers in vivo
CRISPR/dCas9 variants	VP64, p65, VPR	Transcriptional activation
	KRAB, Mxi1, SID	Transcriptional repression by blocking initiation or recruiting repressors
	DNMT3A (full-length or CD)	In vivo DNA methylation and gene silencing
	TET1 catalytic domain	DNA demethylation and transcriptional activation
	G9a, SUV39H1 catalytic domain	H3K9 methylation for gene repression
	LSD1	H3K4me2 demethylation; represses transcription by removing H3K27ac
	p300 catalytic domain	Targeted H3K27 acetylation, ideal for enhancer regions
dCas13	ADAR2, modified ADAR2	RNA editing: adenosine to inosine; cytosine to uracil conversions

Effector domains connected to dCas9 fall into two main types:

1. Transcriptional regulators: recruit additional cofactors, chromatin remodelers, or enzymes.
2. Chromatin modifiers: directly catalyze the addition or removal of epigenetic marks.

Examples include VP16 (and its tetramer version VP64), used to activate gene expression, and KRAB, a repressor domain. To enhance targeting efficiency, the dCas9-SunTag system allows the recruitment of multiple copies of an effector or co-regulators at one site. Additionally, the dCas13 system expands epigenome editing to include RNA modifications, allowing precise RNA-level editing without altering the genome (Adapted from references 15 and 18).

CRISPR-Cas systems differ from zinc finger (ZF) and transcription activator-like effector (TALE) systems in that they are encoded by DNA and guided by RNA, while ZF and TALE approaches depend on protein-DNA interactions to target specific genomic regions [21, 22]. By connecting epigenetic effectors to the deactivated Cas9 protein (dCas9) and directing them toward gene regulatory regions such as promoters and enhancers, CRISPR-based epigenome editing (EpGE) offers greater precision and efficiency [23]. A key advantage of CRISPR systems is that they bypass the need for complex protein engineering to recognize DNA sequences, making them easier to use [24].

Since its development, CRISPR/Cas has received significant attention for its potential in gene-specific epigenetic reprogramming [25]. In the type II CRISPR system, a single-guide RNA (sgRNA) is formed by combining tracrRNA and crRNA, which directs the Cas9 protein to the desired DNA sequence. This sgRNA guides dCas9 to target sequences adjacent to a PAM motif, typically NGG trinucleotide repeats [26]. By changing the gRNA sequence, CRISPR/dCas9 can be programmed to target nearly any location in the genome [27]. Interestingly, multiple gene targets can be edited simultaneously using this approach.

The CRISPR-dCas9 system uses a modified version of SpCas9 that lacks its cutting activity, turning it into a “dead” Cas9 (dCas9). When dCas9 is fused with the catalytic domain (CD) of DNA methyltransferase DNMT3a, it can add methylation marks to specific regions of DNA, silencing gene expression [25, 28]. Similarly, when dCas9 is fused with the core catalytic domain of the p300 acetyltransferase, it promotes the acetylation of lysine 27 on histone H3 (H3K27), which activates gene expression [29].

Depending on the effector it is paired with, dCas9 can either repress or activate gene expression. When connected to repressive domains, it helps silence genes by inducing the formation of heterochromatin. In contrast, when linked to effectors that modify histones (e.g., adding acetyl groups), dCas9 can activate transcription. This system provides a way to direct gene expression patterns that guide cell differentiation and

control developmental pathways. Moreover, CRISPR-dCas9 allows for the reversible repression of many genes, potentially creating heritable epigenetic changes even after the tool is removed [30, 31].

More advanced versions of this system, like the SunTag platform, can target multiple copies of effectors to a single location, increasing editing efficiency. One CRISPR-based EpGE tool, FIRE-Cas9, was even designed to reverse gene modifications if necessary, making the editing process safer and more flexible. This technology has been successfully used to influence the differentiation of human pluripotent stem cells (hPSCs) [21].

Like gene activation systems, repressor modules use DNA-binding proteins fused to silencing effectors. The KRAB (Krüppel-associated box) domain is the most widely used repressor domain **(Table 1)**. KRAB recruits other proteins like KAP1 and enzymes that induce histone methylation and deacetylation, promoting heterochromatin formation and gene silencing. Fusions of KRAB with ZFPs [3], TALEs [3], and dCas9 [32] have shown strong repression capabilities across different genomic elements.

Other effective repressor domains include SID (SIN3 interaction domain) [3] and Mxi1 [9]. Studies have found that dCas9-Mxi1 fusions can achieve nearly triple the repression level compared to dCas9-KRAB [9]. Similarly, TALE repressors fused with SID domains were 26% more effective than those fused with KRAB [3]. Using multiple copies of SID (e.g., SID4X) with DNA-binding proteins enhances repression, similar to how ZFPs are linked to VP64—a tetramer of the VP16 activator—to improve chromatin accessibility [3].

While transcriptional repressors attract proteins to block gene expression by forming heterochromatin, epigenetic effectors such as histone demethylases, histone methyltransferases (HMTs), and DNA methyltransferases (DNMTs) can directly and specifically modify histones or DNA to achieve silencing. ZF, TALE, and CRISPR-dCas9 systems have successfully used these enzymes to strongly suppress gene expression at promoters and enhancers [3, 15, 32].

Recently, RNA modifications have emerged as important regulators of gene expression after transcription [33]. Researchers are uncovering how changes to RNA—both coding and non-coding—affect gene regulation. These modifications help shape gene expression patterns and play vital roles in various biological processes. For instance, the N6-methyladenosine (m6A) mark in RNA is crucial for regulating circadian rhythms, reproduction, embryo development, DNA repair, stress responses, pluripotency, and cell reprogramming [34]. Moreover, m6A regulators are closely linked to cancer development, acting as either tumor suppressors or promoters [35].

Applications of epigenome editing (EpGE)

Epigenome editing (EpGE), especially when designed with inducible systems, is proving to be a powerful tool not only for exploring basic epigenetic mechanisms but also for solving practical real-world problems. Its uses range from agricultural advancements to medical applications [21, 35, 36]. For example, bioengineers have successfully engineered metabolic pathways in algae and corn to produce ethanol, paving the way for sustainable, cost-effective sources of renewable energy. This same technology is helping improve the traits of crops and livestock and is being used in developing new treatments for diseases caused by inherited or acquired epigenetic mutations.

The following sections highlight two key proof-of-concept studies that demonstrate the real-world potential of EpGE.

Epigenome editing in plants

Plant-focused EpGE research is growing rapidly, largely because improving the yield and quality of food crops is vital for global food security. This technology is opening exciting new opportunities in agriculture and horticulture. By investigating epimutations across various crop species, researchers can better understand how epigenetic mechanisms influence critical agricultural traits such as yield, quality, drought tolerance, and disease resistance [16, 22, 36–40].

Epimutagenesis and targeted transcriptional regulation are also being used to study how key proteins interact, modify plant development traits, and shed light on how plants manage, store, and use DNA methylation (DM) [22]. These proteins influence many essential biological processes in plants, including seed development, root and leaf growth, flowering time, and fruit ripening **(Figure 4)**.

This regulation system includes:

- *Writers:* enzymes like methyltransferases that add methyl marks,
- *Erasers:* demethylases that remove those marks,
- *Readers:* proteins that recognize m6A methylation and influence how RNA is processed.

These "readers" bind to methylated RNA and help regulate processes such as RNA splicing, RNA stability, and the function of the RNA's 3' untranslated region (3′UTR) [41].

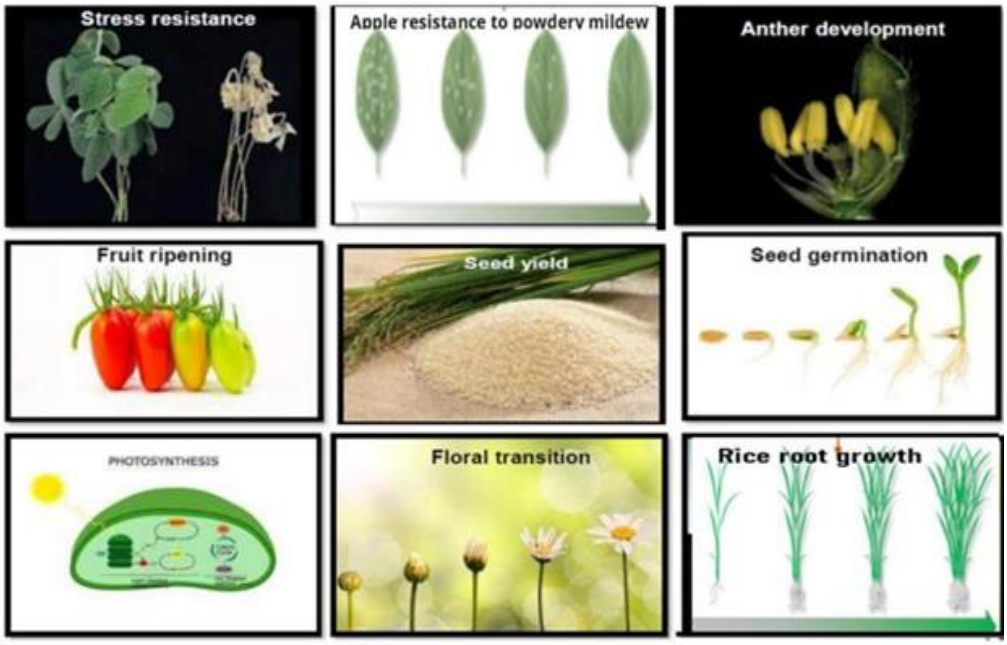

Figure 4. Control of plant growth and development by post-transcriptional changes using m6A, the most prevalent internal modification present in the mRNAs of all higher eukaryotes; post-transcriptional alterations in plants are regulated by various proteins, including "writers" that add m6A, "erasers" that remove it, and "readers" that recognize and interact with m6A-modified RNA; these proteins affect different plant developmental processes such as seed development, leaf and root growth, floral transitions, and fruit ripening [42, 43].

Applications in plant epigenome editing (EpGE)

ZF-based EpGE methods have been applied in *Arabidopsis thaliana* (*A. thaliana*) and *Oryza sativa* (*O. sativa*), alongside the use of TALE and dCas9 systems in these species [41]. Additionally, a variety of small RNA-based EpGE techniques have been used to regulate gene expression across different plants, including *A. thaliana*, *Nicotiana benthamiana*, *O. sativa*, *Solanum tuberosum*, and *Zea mays* [22, 39, 44]. For example, RNA-directed DNA methylation (RdDM) systems using transgenic small interfering RNAs (siRNAs) have been successfully applied in *A. thaliana*, while RNAi-based systems have been used in *O. sativa* [28, 41]. Furthermore, DNA and histone modifications have been achieved through tissue culture-based EpGE in plants like *Caribbean agave angustifolia*, *Henequen (A. fourcroydes)*, *A. thaliana*, *Nicotiana tabacum*, *O. sativa*, *Pinus radiata*, and *Z. mays* [44].

In addition to directly using activator domains to trigger transcription, strategies that modify epigenetic marks to activate gene expression indirectly are also being explored. For instance, the human p300 domain has been utilized in plants to enhance transcription through acetylation of histone H3 at lysine 27 (H3K27ac). Another method involves the plant-specific HAC1 domain from *Arabidopsis*, which can increase gene transcription. However, VP64 is more effective, particularly for genes like p300 [45]. Combining multiple activator domains to act synergistically has proven to enhance gene expression, with combinations like VP128 and TAL activator domains significantly boosting gene activation [45, 46]. Similarly, pairing VP64 with other effectors such as P65 and Rta has been shown to increase gene expression in plants more than VP64 alone [45, 46]. For gene repression in plants, the most common epigenetic effectors are those found in *Arabidopsis* ethylene response factors, such as SUPERMAN and BODENLOS. These domains are often used to study genes with redundant functions and can override activator domains to turn them into repressors [47].

Additionally, DNMTs, like those from *Nicotiana tabacum*, can be used for adding DNA methylation to specific promoters, thereby repressing transcription [48]. In addition to using epigenome editing for repression, CRISPR interference (CRISPRi) is a method that directly suppresses gene expression by inhibiting RNA polymerase II activity. Although CRISPRi has been successfully applied in plants, it has only been reported in *Zea mays* (maize) to partially suppress a gene [43]. To date, no studies have explored the combination of multiple repressor domains to suppress transcription in plants [22].

Epigenome editing in medicine

There is growing evidence that alterations in the genome (genetic mutations) and the epigenome (epi-mutations), or both, are closely associated with the development of several diseases in humans, such as cancer, type II diabetes, neurological disorders, cardiovascular diseases, and even psychiatric conditions [8, 9, 14, 49, 50]. The epigenome, which connects the genome to environmental factors, is dynamically modified throughout the progression of many diseases **(Figure 5)**. Understanding these epigenetic changes before disease onset could lead to the development of targeted strategies to prevent chronic diseases. As more research uncovers the role of epigenetic processes in disease, scientists are working to identify epigenomic changes that regulate genes involved in cell growth and immune function. Additionally, epigenetic markers are being explored as potential tools for detecting disease risk factors.

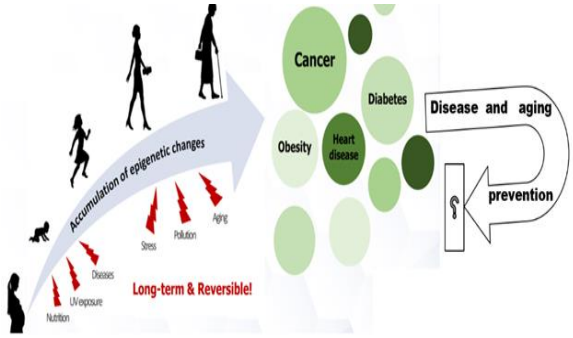

Figure 5. Environmental factors, acting at various moments throughout the life cycle, can result in epigenetically mediated alterations in gene expression and consequently in phenotype. Potential public health intervention may become a reality (The idea inspired from: [51]).

Targeted epigenetic regulation in medicine

In cases where traditional gene therapies are either unavailable or not appropriate, targeting disease-related genes could present a new avenue for treating a wide range of disorders [18]. While the potential effects on population health and across generations are not fully understood, these approaches are becoming increasingly important in the fields of applied genomics and personalized medicine [17]. Recently, epigenetic drugs and biomarkers have entered clinical trials targeting various cancers, with several already receiving FDA approval for treating conditions like myelodysplastic syndromes and leukemias [35, 49]. It appears that different transcriptional activators can induce gene expression with varying levels of intensity [3].

One such activator is the farnesyl pyrophosphate synthase (FPPS) enzyme, located in the mevalonate pathway, which has been linked to transcriptional activators like the p65 component of the NF-kB complex **(Table 1)**. This enzyme is a critical therapeutic target, with recent studies identifying a druggable site near its active region, though its exact biological function remains unclear [52].

Research focusing on the maspin promoter region has successfully reactivated the maspin tumor suppressor gene, often silenced in aggressive cancers [53]. This highlights the potential of ZFP-based EpGE in treating neurodegenerative diseases [54]. For example, when TALE activators were directed at the promoter region of the human vascular endothelial growth factor-A (VEGF-A) gene, which plays a role in tumor blood vessel growth, gene expression increased by up to five times [11]. Similarly, combining TALE fusions with the VP64 activator resulted in a two to five-fold increase in the expression of pluripotency factors in human cells **(Table 1)**. These combined efforts suggest that manipulating these activators could create powerful, adjustable transcriptional networks for therapeutic use [11].

Figure 6 presents diseases that could benefit from epigenetic therapies, especially neurological conditions. For instance, fragile X syndrome, a leading cause of intellectual disability in males, is caused by the silencing of the FMR1 gene through abnormal DNA methylation. Using a technique that precisely demethylates the FMR1 promoter with the dCas9-TET1 system has successfully reactivated gene expression in lab-grown neurons [55]. Likewise, similar methods have shown success in treating imprinting disorders like Prader-Willi syndrome

(PWS) and Angelman syndrome (AS), which result from irregular DNA methylation [19].

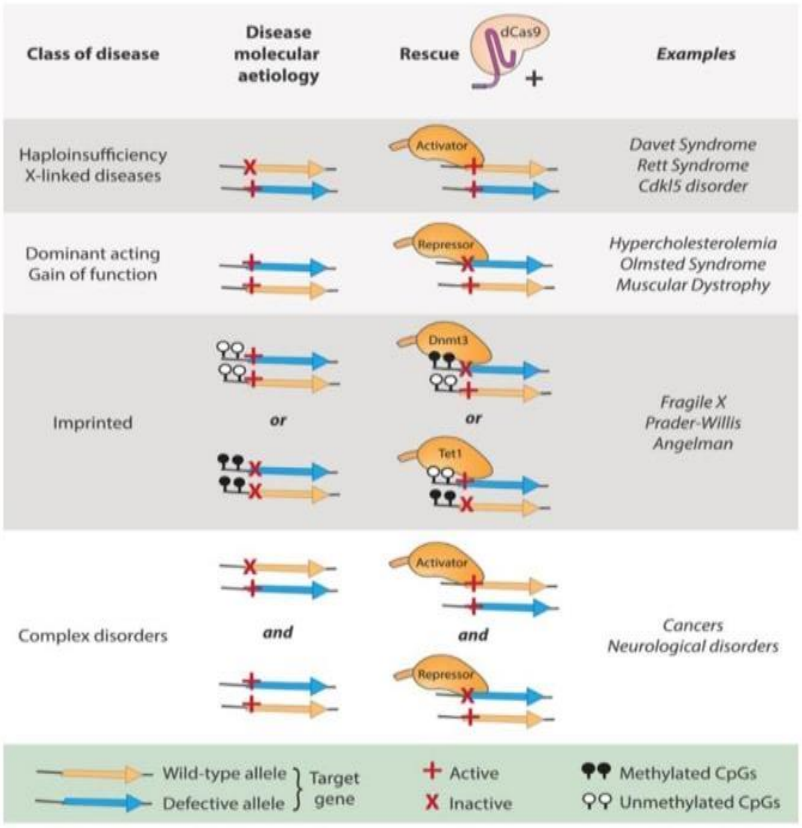

Figure 6. Prospective therapeutic applications of EpGE; different categories of disease could benefit from the development of discrete CRISPR/Cas9-driven EPGE strategies; examples of how mutant or wild-type alleles could be maneuvered in specific disease contexts are depicted here; the molecular etiology underlying each disease class, as well as the dCas9-based rescue strategy, are present [19].

Targeted epigenetic regulation in pain and inflammatory disorders

Epigenetic approaches, such as targeting specific cytokines in the inflammation pathway, offer a promising strategy for treating degenerative disc disease [56] and a range of pain-related conditions by influencing cutaneous pain receptors [57]. Surprisingly, diseases like Olmsted syndrome, which affects the skin, present a unique opportunity for CRISPR-based gene silencing techniques, making the skin an accessible target for therapeutic intervention.

Recent research has also highlighted the molecular mechanisms behind m6A RNA modifications, revealing how variations in these modifications are linked to several disorders, including cancer. Additionally, modifications to tRNA, such as m5C and mcm5U, are now recognized for their role in cancer progression and their potential to alter protein translation processes [58]. Unlike traditional genetic editing, EpGE, like RNA editing, avoids the complications of permanent genetic modifications. For instance, the use of CRISPR-dCas9 to silence the SCN9A gene, which encodes a sodium channel involved in pain sensation, has demonstrated therapeutic potential in treating chronic pain in mouse models [59]. This method opens the door to targeting reversible epigenetic changes as a way to treat a variety of conditions, including cancer, diabetes, and neurodegenerative diseases [24].

Cancer and neurodegenerative disease treatment

Epigenetic therapies also show promise in addressing neurodegenerative diseases. Recent studies have highlighted how such therapies can reduce tau protein

levels, which are implicated in neurodegenerative conditions like Huntington's disease [60, 61]. Moreover, epigenetic changes are strongly associated with cancer, and utilizing CRISPR/Cas-based EpGE systems offers the potential to simultaneously activate tumor suppressor genes and silence oncogenes [8, 24, 54, 62]. By combining transcriptional regulators with CRISPR, researchers have succeeded in simultaneously turning on and off multiple genes within a single cell [59].

Limitations and challenges in EpGE

Despite its potential, EpGE faces several challenges. Current methods have shown strong correlations between epigenomic modifications and gene regulation but fall short of proving causality. There is also a need for more precision in EpGE, as sequence specificity is crucial for its success. Another hurdle is the lack of standardization across new products, including finding appropriate transformation vectors and suitable promoters for cloning. One of the major challenges for EpGE, especially in humans, is effectively delivering the system into target cells. Although several delivery methods for large dCas9 fusion proteins have been identified [20, 26, 56, 63-78], significant obstacles remain in their application for in vivo studies.

Moreover, the interaction between TALE combinations and epigenome-modifying proteins remains unclear, especially regarding their potential impact on catalytic activity. In cases where multiple subunits are required, interference by other proteins targeting the same sequence may affect the desired outcomes [51]. Additionally, off-target effects pose a significant risk, particularly when suboptimal sgRNAs or dCas9 fusion proteins are used, which could hinder the desired epigenetic modifications from taking hold.

In plants, one challenge is the reduction of off-target mutations and the need for faster generation times for more efficient application of these tools. In some cases, eliminating the editing reagents may require multiple generations, making transient expression methods particularly valuable. Advanced sequencing techniques such as MeRIP-seq and miCLIP may assist in mapping m6A modifications at the cellular level [49].

Conclusion

The discovery and adaptation of the CRISPR-Cas9 system have significantly improved the ease of conducting gene editing (GE) and epigenetic gene editing (EpGE). Through the use of CRISPR/dCas9, EpGE has become an efficient targeted approach for potential applications in precision medicine. The fusion of chromatin-modifying domains with dCas9 has enabled targeted gene activation or repression in both cultured cells and in vivo animal models. However, despite the remarkable advances in understanding epigenetic processes, EpGE remains an evolving field with several unresolved challenges. These include off-target effects, editing efficiency, delivery mechanisms, cytotoxicity, specificity, and the stability of epigenetic changes.

It is expected that future developments, such as a conformationally activated CRISPR/Epi-editor, where epigenetic enzymatic domains are integrated with Cas9's nuclease domains, could offer better precision and fewer off-target effects, opening the door for potential therapeutic applications in humans. Additionally, controlling the expression of dCas9 fusion proteins through an inducible promoter could help reduce off-target effects by regulating their production.

In clinical settings, ongoing pre-clinical and clinical trials are evaluating epigenetic drugs, either alone or in combination with other treatments. A significant challenge is overcoming resistance to conventional epigenetic drugs and expanding their therapeutic use beyond hematological malignancies. Innovative approaches are therefore urgently needed. One promising strategy is to use epi-drugs before chemotherapy to make cancer cells more sensitive by enhancing chromatin accessibility. The combination of patient-derived iPSCs with EpGE technology allows for more accurate disease modeling and a deeper understanding of how epigenetic marks contribute to disease progression. "Programmable" EpGE technologies are being developed to target specific genomic loci, enabling the study of their function in different cellular contexts. Developing effective delivery methods to target all disease-related cells in cancer therapy remains a challenge.

Lastly, the discussion around EpGE would be incomplete without addressing its ethical and moral implications. While EpGE does not directly alter the genome and is believed to have less impact on germ cells than traditional gene editing, the possibility of transgenerational epigenetic inheritance raises concerns. There are questions about the health and safety of epi-engineered crops and food products, as well as concerns over whether epigenetic changes could be used as biomarkers for disease susceptibility later in life.

Additionally, the potential for discrimination based on epigenetic data is a serious concern. As a result, there are growing calls for policies to regulate the use of private epigenetic and genetic information, especially for non-medical purposes.

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Ethics Statement: None

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