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INVITED REVIEW PAPER

Epigenome editing and epigenetic gene regulation in disease phenotypes

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Abstract−Proper gene control across space and time is crucial for the seamless execution of various cellular functions. Rapid advancements in genome-wide studies revealed that in addition to genetic mutations, epigenetic modifications also play an important role in cellular processes and disease development. Epigenetic modifications, including DNA methylation and post-translational modifications on histones via methylation, acetylation, phosphorylation, etc., do not alter DNA sequences. Yet, disruptions of the epigenome can still induce gene malfunction, aberrant cell differentiation, proliferation, and apoptosis, resulting in various diseases such as cancer, neurological disorders, and autoimmune diseases. This review describes the association between epigenetic modifications and disease phenotypes, current techniques to perturb the epigenome and analyze changes in gene expression, and perspectives on future epigenetic research.

Keywords: Epigenetics, Gene Regulation, CRISPR-dCas9, DNA Methylation, Histone Modification

INTRODUCTION

Transcriptional regulation is a key process in development, as too much or too little expression in the wrong place or time leads to developmental defects and disease phenotypes. For example, cancerous tumor growths are often associated with the overexpression of the *Myc* oncogene, which causes uncontrolled cell proliferation [1]. In another context, lactose intolerance is caused by a single point mutation in the regulatory DNA of the lactase *LCT* gene, which significantly reduces enzyme production [2]. Correcting such faulty gene expression requires a systematic understanding of the factors that affect the gene regulatory network. Many disease phenotypes arise from point mutations in the coding region of a gene that change the amino acid sequence and disrupt protein functions. Recent studies, however, emphasize that epigenetic modifications also have a major impact on disease development [3].

Epigenetic modifications refer to physical and conformational changes to the genome that affect gene expression without altering DNA sequences. These changes include DNA methylation and post-translational modifications on histones via methylation, acetylation, phosphorylation, etc. It was shown that epigenetic modifications often cause various types of gene malfunctions, which are highly associated with disease phenotypes. In this review, we summarize known associations between epigenetic modifications and diseases, introduce techniques that have been developed to perturb the epigenome, and provide our perspectives on where future epigenetic research is headed.

1. Epigenetic Modifications and Disease Phenotypes

To date, a great number of epigenetic modifications on the genome have been linked to various disease phenotypes, including developmental and neurodegenerative disorders, cancers, and autoimmune diseases. Here, we present a few diseases known to be caused by misregulation of the epigenome. Fig. 1 summarizes different types of epigenetic modifications and associated diseases (Fig. 1).

1-1. Neurological Disorders

Fragile X syndrome (FXS) is an inherited neurological disorder where the role of epigenetic regulation on disease phenotypes is well characterized. Affecting about 1 in 7,000 males and 1 in 11,000 females, FXS causes various types of intellectual and developmental disabilities [4]. FXS is caused by the expansion of a CGG repeat located in the 5′ UTR of the fragile X mental retardation 1 gene (*FMR1*). While individuals typically carry 6-44 CGG repeats within the *FMR1* locus, those with FXS are shown to have 200 or more CGG repeats in the locus. Such highly repeated sequences induce abnormal DNA methylations in the promoter region, such that about 65% methylation level was observed at the *FMR1* promoter in FXS-affected human embryonic stem cells (ESCs) compared to the 2% methylation level in wildtype human ESCs [5-7]. *FMR1* hypermethylation is often observed in undifferentiated cells, and it also accompanies histone modifications such as H3K4me3 downregulation and H3K9me3 upregulation - markers for active transcription and heterochromatin, respectively. In FXS-affected human ESCs, the H3K4me3 mark was decreased by more than five-fold, whereas the H3K9me3 mark was upregulated by more than threefold [8,9]. As a result of such DNA methylation and histone modifications, the *FMR1* gene is silenced in FXS cells, resulting in a significant reduction of the FMR protein (FMRP) [10].

Another neurological disorder that is caused by epigenetic modification is Rett syndrome, a rare neurodevelopmental disorder that disrupts brain development, especially among females. Rett syndrome is mainly caused by genetic mutations in the methyl CpG binding protein 2 (*MeCP2*) locus [11], which encodes MeCP2 protein, an epigenetic regulator that binds to methylated DNAs and represses the gene. Most missense mutations from Rett syndrome

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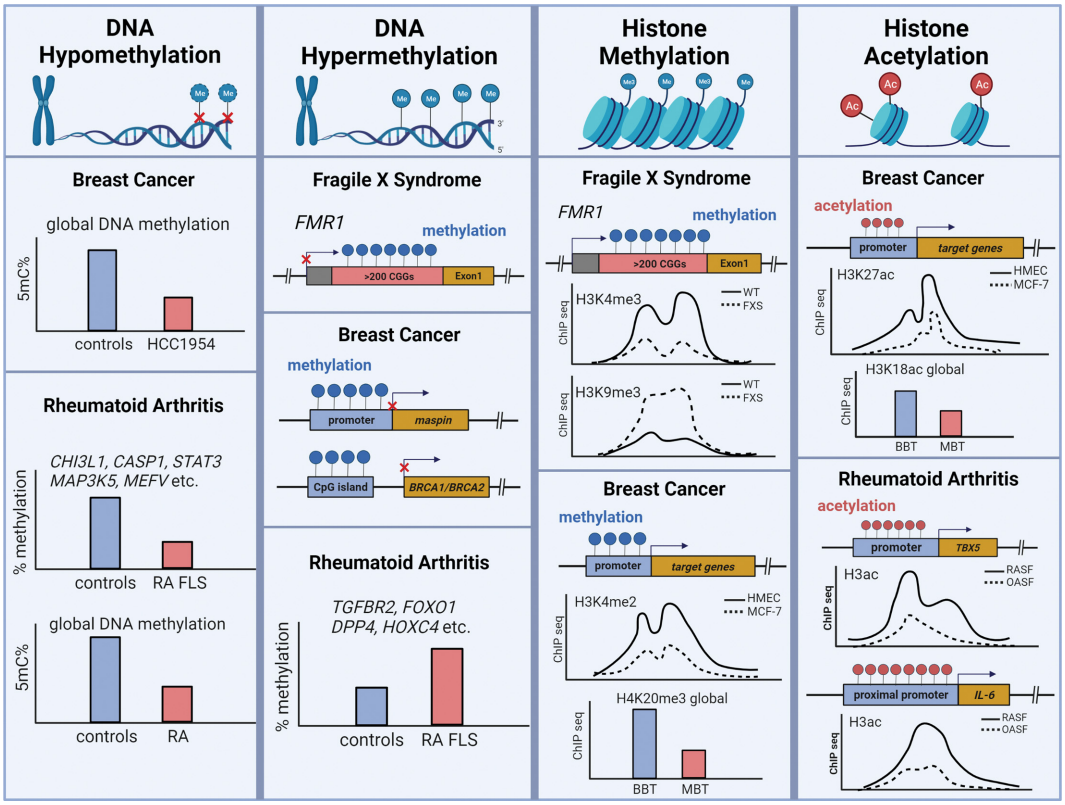

Fig. 1. Different types of epigenetic modifications with known associated diseases. Each column represents the type of epigenetic modifications and diseases associated with each modification, such as DNA hypomethylation, DNA hypermethylation, histone methylation, and histone acetylation. Under each disease, changes in epigenetic marks between diseased and control tissues are illustrated.

patients are found in the methyl binding domain of *MeCP2*. These mutations disrupt the MeCP2 protein's ability to interact with methylated DNAs during neuronal development, affecting transcriptional regulation of key neural genes [12]. This is a case where genetic mutations of a gene have an epigenetic effect of changing chromatin landscape, inducing disease phenotypes.

Often, disease phenotypes arise from misregulation of multiple genes, rather than a single gene. When Alzheimer's patients' brains were sequenced, more DNAs across the genome were in methylated states compared to the normal brain tissues. Many hypermethylated sites were observed within CpG islands, which are often located near promoters of protein-coding genes in the human genome. Methylation of CpG islands results in silencing of the nearby target gene, suggesting that expression of many brain function genes associated with Alzheimer's pathology is disrupted by DNA hypermethylation. Such differentially methylated regions are also correlated with other repressive epigenetic markers, such as H3K27me3 and H3K9me3 [13]. In addition to the three disorders mentioned in this review, an increasing number of studies have identified correlations between epigenetic modifications and neurological disorders, including Huntington's disease, Parkinson's disease, and autism [14].

1-2. Cancers

Breast cancer is a common cancer detected predominantly in women, with more than 250,000 reported cases per year in the United States [15]. More recent papers reveal that epigenetic modifications, including DNA methylation and post-translational histone modifications, play an essential role in breast cancer development. Downregulation of many genes like *BRCA1*, *BRCA2*, and *SERPINB5*, are considered to be risk factors of breast cancer, and hyper DNA methylation and histone modifications were found to affect the transcription of these key oncogenes [16,17].

Maspin, which is encoded by *SERPINB5*, works as a tumor suppressor in cancer cells. Maspin is expressed in normal breast cells, but its expression is reduced or lost in breast cancer cells. Futscher et al. showed that Maspin expression was lost among ~57% of ductal carcinoma in situ specimens [18]. Among many factors that resulted in Maspin silencing, DNA hypermethylation at the Maspin promoter was found to be a key factor [19]. DNA hypermethylation indirectly blocked transcription factors binding to their cognate sites at the promoter region, resulting in downregulation of the gene [18].

Breast cancer gene (*BRCA*) family comprises other well-known tumor suppressors, which repair DNA breaks. Mutations in *BRCA* genes prevent the repair of DNA damage and lead to uncontrollable tumor growth [20]. Often, patients without any germline mutations in the *BRCA1* or *BRCA2* locus show reduced BRCA1/BRCA2 protein levels. Such a decrease seems to be associated with DNA hypermethylation in cancer tissues. Aberrant DNA methylation of the CpG island on the promoter region of *BRCA1* and *BRCA2* was confirmed by methylation-specific PCR. The *BRCA* promoter methylation level in malignant breast tumors was four-fold higher than

in benign breast tumors [16]. Indeed, the level of *BRCA* expression was comparable between the tissues with hypermethylated *BRCA1* locus without genetic mutations and the *BRCA1-null* tissues with mutated *BRCA1*, emphasizing the effect of epigenetic modifications on cancer development [21].

Recently, studies with reduced-representation bisulfite sequencing (RRBS-seq) showed that aberrant DNA methylation was observed throughout the genome in multiple cancer tissues, affecting the activity of hundreds of promoters. Many of the aberrant methylation sites were correlated with gene expression, such that highly methylated sites either upregulated or downregulated key tumor suppressors and oncogenes [22]. Interestingly, some reported a correlation between DNA hypomethylation and epigenetic changes. Unlike the previous assumption that DNA hypomethylation leads to gene activation, Hon et al. demonstrated that the global DNA hypomethylation in breast cancer cells is associated with the formation of repressive chromatin domains and results in gene silencing [23].

In addition to DNA methylations, histone modifications were observed within or near transcription start sites of the upregulated and downregulated genes in human breast cancer cells (MCF-7) compared to normal human epithelial cells (HMEC). Active transcription markers H3K79me2, H3K27ac, and H3K4me2 showed the most significant downregulation near transcription start sites, suggesting that these three histone marks may result in aberrant gene expression in breast cancer cells. Furthermore, many histone marks were positively or negatively correlated with others, indicating that these modifications could jointly regulate gene expression levels in breast cancer cells [24]. Similarly, Paydar et al. compared the global level of three different modifications between malignant breast tumors (MBT) and benign breast tumors (BBT) and discovered notably low levels of H3K18ac and H4K20me3 in patients with MBT [16]. Similar genome-wide epigenetic modifications were observed in many cancer cells, including bladder, colon, gastric, liver, and lung, where these changes disrupted transcriptional programs and affected tumor progression [25]. Taken together, these studies all emphasize the importance of further analyzing the relationships between various epigenetic modifications and cancers.

1-3. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease in which the immune system attacks its own joint tissues and causes pain. Many factors are responsible for RA, including genetic mutations, epigenetic dysfunctions and other environmental factors [26]. Of such, an increasing number of studies demonstrate the role of epigenetic modifications in RA pathologies. T cells derived from RA patients demonstrated global DNA hypomethylation, where many promoters exhibited lower methylation levels than normal T cells [27]. A comprehensive DNA methylation profile generated by Nakano et al. revealed hypomethylation in novel RA-related genes such as *CHI3L1*, *CASP1*, *STAT3*, *MAP3K5*, *MEFV*, and *WISP3* in RA Fibroblast-like synoviocytes (FLS), which all led to more loosely packed or open chromatin conformation and resulted in upregulation of the genes [28]. While more hypomethylated regions were identified throughout the genome, some loci exhibited higher DNA methylation levels. DNA hypermethylation occurred in *TGFBR2*, *FOXO1*, *DPP4*, and *HOXC4* promoter loci, and such hypermethylation was associated with decreased expression of corresponding genes [28,29].

Aberrant histone modifications also play a role in the pathogenesis of RA. Histone active mark H3K4me3 and histone 3 acetylation (H3ac) mark were highly enriched in the promoter region of *TBX5*, a transcription factor that is highly expressed in RA synovial fibroblasts (RASFs), whereas H3K27me3 level was downregulated in RASFs compared to the osteoarthritis synovial fibroblasts (OASFs) [30]. Reduced H3K27me3 level was also observed in the promoter region of *SFRP1*, a *Wnt* inhibitor that is associated with the activation of RASFs [31]. H3ac level was also significantly higher in the IL-6 promoter in RASFs. IL-6 is known to be involved in the pathogenesis of RA and its production was remarkably increased in RASFs. The more pronounced H3ac enhancement occurred near the proximal IL-6 promoter than the distal IL-6 promoter [32].

2. CRISPR-dCas9 System to Edit Epigenome

Given the close correlation between epigenetic modifications and various disease phenotypes, extensive studies have examined the correlation between epigenetic modification and transcriptional regulation. Techniques developed in the past decade enable the regulation of chromatin states without changing the corresponding DNA sequences. Of many such techniques, we will focus on CRISPR/dCas9-mediated epigenome editing, an advanced genome editing tool with high efficiency and accuracy. CRISPR technology was initially developed to induce double-strand breaks to a specific genomic locus and edit DNA sequences with high precision [33,34]. In the Type II CRISPR-Cas system, Cas9 nuclease is guided to a specific locus by a single guide RNA (sgRNA) consisting of a 20-30 bp targeting sequence, and cleaves the DNA ~3 bp upstream of a protospacer adjacent motif (PAM) site. By designing the homology-directed repair template consisting of specific gene modifications flanked by left and right homology arms, precise gene editing can be performed in a site-specific manner [35]. CRISPR-Cas9 system achieves genome editing with high efficiency, high specificity, and design simplicity compared to other genome editing techniques, and hence the technique has been used across many tissue types and model organisms [35].

The CRISPR/dCas9 system was developed to utilize the property of the CRISPR system without inducing permanent changes in the genome. The technique utilizes catalytically dead Cas9 (dCas9), which contains two point mutations on the residues (i.e., D10A and H840A). dCas9 is still capable of recognizing the target DNA sequence by being recruited to the sgRNA, but it cannot cleave the target DNA [36]. dCas9 can be fused with multiple repressor and activator proteins and recruited to the promoter region to repress or activate the target gene without changing the genomic DNA [36,37]. Recently, dCas9 was fused with multiple epigenetic regulators to perturb the epigenome. In this section, we discuss recently developed CRISPR-dCas9 systems targeting different epigenetic markers and provide examples of how these techniques are utilized to perturb the epigenome in disease models mentioned earlier.

2-1. DNA Methylation

The CRISPR/dCas9 system has been used to study the role of DNA methylation on Fragile X Syndrome (FXS). Induced pluripotent stem cells (iPSCs) and ESCs derived from FXS patients were used as a model system to investigate the methods to reduce the

disease phenotypes through epigenetic editing. To reactivate *FMR1* expression in FXS iPSCs, DNA demethylation was induced at the hypermethylated region of the *FMR1* gene by fusing dCas9 with the catalytic domain of a demethylase Tet methylcytosine dioxygenase 1 (TET1CD) and designing sgRNAs targeting the CGG repeat region [7,38]. As a Ten-eleven translocation (TET) family, TET1 is involved in DNA demethylation and downstream gene regulation. The cells transduced with both dCas9-TET1CD and CGG sgRNAs were able to restore up to 90% of the *FMR1* expression in the WT iPSCs. Histone repressive marks (H3K9me3) were also significantly reduced and active histone marks (H3K4me3 and H3K27ac) were increased at the CGG repeat region in dCas9-TET1CD transduced cells. This indicates that the dCas9-TET1CD targeting the CGG repeat region is sufficient to demethylate the hypermethylated region of the *FMR1* gene in a site-specific manner. Similar results were obtained when the dCas9-TET1CD and sgRNA-CGG-transduced neuronal precursor cells were implanted into the P1 mouse brain, suggesting that the CRISPR/dCas9-mediated *FMR1* reactivation occurs in vivo as well [7].

In addition to the *FMR1* locus, the dCas9-TET1CD was used to induce site-specific demethylation at other hypermethylated regions, both in vitro and in vivo. Choudhury et al. recruited dCas9-TET1CD fusion protein to the *BRCA1* promoter region to demethylate the hypermethylated DNAs in breast cancer cells [39]. The assay successfully demethylated the region and resulted in upregulation of *BRCA1* transcriptional activity, providing a useful assay to perturb the epigenome at various tumor suppressor gene loci. In Morita et al., dCas9 was fused to a repeat peptide GCN4, which recruits anti-GCN4 peptide antibody (scFV). TET1CD was fused to scFV, and that way, multiple copies of TET1CDs were recruited to a sgRNA target locus and dCas9. In mESCs, a significant increase in DNA demethylation was reported in the presence of both dCas9-GCN4 and scFV-TET1CD fusion proteins, resulting in a notable upregulation of target genes. This technique was successfully applied to the brain of mouse fetuses, providing a potential therapeutic cue [40].

Similarly, DNA methyltransferase inhibitors (DNMTi) are commonly used as epigenetic and therapeutic drugs to block DNA methylation. DNMTi's like azacytidine and 5-aza-2′-deoxycytidine were used to activate silenced genes by trapping or blocking DNA methyltransferases [41,42]. Recent clinical trials and experimental studies revealed that the treatment combining DNMTi and antitumor drugs showed high effectiveness and low toxicity [41]. Notably, recent study demonstrated that dCas9 and sgRNA alone without other epigenetic modifier proteins could physically hinder DNA methylation at specific target sites [43].

dCas9 system was used to methylate the unmethylated DNA regions as well. Vojta et al. designed a CRISPR-dCas9 system by fusing dCas9 with the catalytic domain of DNA methyltransferase DNMT3A (DNMT3ACD) to induce DNA methylation at specific sites. Different sgRNAs were designed to target the *BACH2* promoter region in HEK293 cells. The most efficient sgRNA was able to increase the methylation level at the *BACH2* locus by up to 35% with individual sgRNAs and 65% with pooled sgRNAs. A two-fold decrease in *BACH2* gene expression was observed, demonstrating the feasibility of the CRISPR-dCas9-DNMT3ACD system to induce gene repression by increasing DNA methylation level [44]. Similar results were obtained using the fusion of dCas9 with other methyltransferases, such as DNMT3B and DNMT3L, while the catalytic domain of DNMT3A was the most efficient [45,46].

2-2. Histone Modifications

Post-translational histone modifications regulate chromatin structures and are involved in various biological processes. Chromatin states are implicated by either histone active marks (e.g., H3K4me3 and H3K27ac) or histone repressive marks (e.g., H3K9me3 and H3K27me3). Multiple enzymes were identified to mediate histone methylation and were utilized in the CRISPR-dCas9 system to modify chromatin states [47,48]. Histone acetylation is a necessary modification that regulates cell cycle proliferation and differentiation. Histone deacetylase (HDAC) removes acetyl groups from histone proteins, which in turn makes the genome less accessible to transcription factors. Therefore, HDAC enzymes fused with dCas9 could achieve site-specific histone deacetylation to repress loci of interest. For instance, dCas9-HDAC3 fusion protein successfully deacetylated histones and induced moderate gene repressions at the targeted promoter regions of *Smn1*, *Mecp2*, and *Isl1* in murine N2a cells. The choice of a sgRNA was critical for dCas9-HDAC3 function, such that sgRNAs needed to be positioned adjacent to H3K27ac marks [49].

Similarly, dCas9 can be fused with the core domain of a histone acetyltransferase p300 (p300core) to acetylate histones and activate target genes. The dCas9$^{p300\ core}$-mediated H3K27 acetylation was enhanced significantly at the targeted *IL1RN*, *MYOD* and *OCT4* promoter regions with sgRNA and resulted in transcriptional activation. The authors compared the level of gene activation driven by dCas9^{p300} and dCas9^{VP64}, which is generated by fusing dCas9 to a transactivator VP64. Upon being directed to a specific locus by sgRNA, dCas9^{VP64} can activate the target gene without affecting the epigenome [50]. The authors showed that dCas9$^{p300\ core}$ fusion protein outperformed the dCas9^{VP64} due to its increased transactivation capacity and higher specificity [51]. Numerous therapies in breast cancer have been developed based on epigenetic mechanisms as well [52]. Histone deacetylase inhibitors (HDACi) have been most commonly used to demethylate CpG islands near tumor suppressor genes and activate gene expression. In fact, DNMTi and HDACi have been shown to work synergistically to activate a silenced gene. In clinical trials, other therapies, including endocrine therapy and chemotherapy, are often combined with DNMTi and HDACi treatment, emphasizing the role of epigenetic regulations in cancer treatment [52].

2-3. Enhanced CRISPR-dCas9 System for Inducible and Reversible Epigenetic Modifications

CRISPR/dCas9-mediated epigenome editing has been improved over the years, and recent studies have provided an assay where chromatin states can be modulated in an inducible and reversible manner (Fig. 2). To achieve temporal control of chromatin modifications, Braun et al. developed the Fkbp/Frb inducible recruitment for epigenome editing by Cas9 (FIRE-Cas9) system that consists of four components: (1) a modified sgRNA containing two MS2 loops, (2) dCas9, (3) a chromatin regulator of interest tethered with Frb, and (4) Fkbp fused with MS2 coat protein (MCP). Fkbp-MCP binds to the MS2 loops in sgRNAs, which provides locus specificity. Upon rapamycin treatment, dimerization between Frb and Fkbp

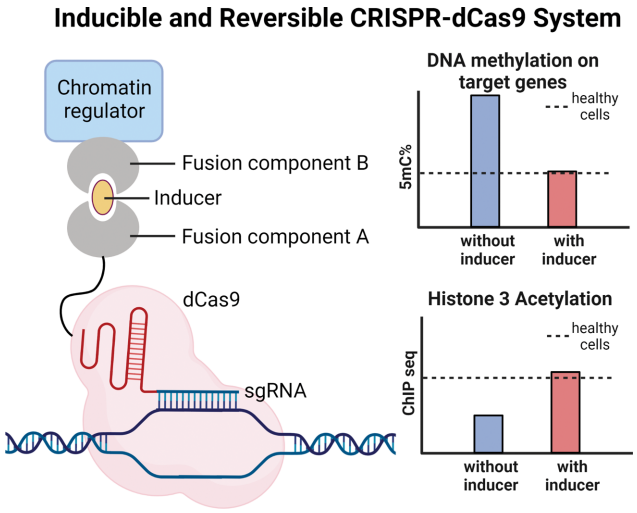

Fig. 2. A reversible and inducible CRISPR-dCas9 system for epigenetic regulation. (Left) Schematic of the dCas9 system for epigenetic regulation. dCas9 is fused with the component A, and a chromatin regulator is fused with the component B. Treating with an inducer allows the fusion of the chromatin regulator and dCas9. Directed by the designed sgRNAs, the chromatin regulator can be brought to a specific target locus and induce epigenetic changes. (Right) Bar charts that qualitatively show the changes in chromatin states with or without an inducer. Dashed lines show the level of chromatin marks extracted from healthy cells.

recruits the chromatin regulator to the target loci, inducing the intended chromatin modification. The authors fused Frb with the heterochromatin protein 1 (HP1), and expressed dCas9 and sgRNAs targeting three loci upstream of the highly expressed *CXCR4* gene in human HEK293 cells. A significant H3K9me3 deposition was observed at the recruitment sites and up to 90% reduction in *CXCR4* expression was observed after five days of the rapamycin treatment, indicating the gene-silencing ability of HP1 complex using the FIRE-Cas9 technique. After the rapamycin wash-out, the gene expression level returned to normal, suggesting the reversibility of the FIRE-Cas9 system [53].

Similarly, Gao et al. developed a reversible and inducible assay called CRISPR-engineered chromatin organization (CRISPR-EChO) adapted from an abscisic acid (ABA)-dCas9 design [54,55]. Two fusion proteins include (1) dCas9 fused to the ABI domain and (2) HP1α-sfGFP fused to the PYL1 domain (Fig. 2). In the presence of abscisic acid (ABA), colocalization between the PYL1-sfGFP-HP1α fusion protein and the sgRNA target loci was observed, indicating successful recruitment of HP1α to the target locus. Three distal genes near the sgRNA target site exhibited a moderate to significant downregulation [55]. While the CRISPR-EChO system allowed the study of higher-order chromatin organization in a reversible and inducible manner with precise temporal and spatial resolution, no significant enrichment of heterochromatin markers like H3K9me3 or KAP1 was observed. This raised further questions on how HP1α interacts with other effectors to form heterochromatin.

Lastly, some recent studies reported the use of optogenetics in combination with the genome editing system to edit epigenome [56]. Lo et al. used the CRY2-CIB1 optogenetic pair, which interacts with high affinity under blue light [57]. CRY2 was fused with either DNMT3A-CD or TET1CD to modify the DNA methylation state, and target specificity was obtained by transcription activation like element (TALE)-CIB1 fusion protein. TALE was used to target the *Ascl1* promoter region. The rat dorsal root ganglion (DRG) neural stem cells (NSCs) co-transfected with DNMT3A-CD-CRY2 and TALE-CIB1 fusion proteins exhibited increased methylation levels at differential methylated regions (DMRs) in the *Ascl1* locus upon blue-light illumination, and *Ascl1* expression level was reduced about 40%. Similarly, demethylase TET1-CD-CRY2 and TALE-CIB1 fusion proteins were co-transfected to the striatal (STR) NSCs, where the *Asd1* region is highly methylated. Under blue-light exposure, transfected STR NSCs revealed a significant decrease in methylation at the *Ascl1* promoter, resulting in an increase in *Ascl1* expression [56]. Although no study has yet combined optogenetic tools with the CRISPR/dCas9 system to edit the epigenome, this study with TALE presented great potential for optogenetic-mediated epigenome editing.

3. Future Perspectives on Timescales of Epigenetic Modification and Gene Expression

While extensive studies have advanced the field of epigenetics in the past decade, many underexplored areas still remain, mainly due to technical limitations. One area that needs to be addressed is the timescale at which epigenetic changes occur and the timescale at which the epigenetic modifications affect downstream gene expression. To address this, fluorescent live imaging can be used to visualize both the epigenetic states as well as the transcriptional activity.

A few biosensors have been developed to detect epigenetic signals at a specific genomic locus in living cells. Lungu et al. and Hori et al. used an engineered DNA methyl binding domain fused with fluorescent tags to visualize DNA methylation in living cells [58,59]. Lungu et al. used bimolecular fluorescence complementation (BiFC)-based bimolecular anchor detector (BiAD) sensor to detect a specific chromatin mark [58]. The sensor consists of an anchor module recruited to a specific DNA locus, and a detector module that binds to a specific chromatin state. The anchor module utilizes CRISPR/dCas9 system to achieve specificity, and the detector module uses methyl binding domains or HP1b that recognizes DNA methylation and H3K9me3, respectively. Each module contains a partial Venus fluorescent protein, and the close proximity between the anchor and the detector modules allows the reconstitution of an intact Venus, emitting fluorescent signals. As a result, an epigenetic marker of interest at a specific locus can be visualized using this biosensor.

Such epigenetic modifications can be correlated with transcriptional activity using live-imaging methods of nascent transcript detection. Most commonly used methods utilize the binding between the MS2 stem loops and MS2 coat proteins (MCP) and PP7 stem loops and PP7 coat proteins (PCP) [60,61]. Derived from bacteriophages, 10-24 copies of MS2 or PP7 sequences can be inserted into the 5′ or 3′ UTR of the gene of interest using CRISPR-mediated genome editing. Upon transcription, the MS2 or PP7 sequences form a stem loop structure, where each stem loop can be bound by two copies MCP or PCP fused with a fluorescent protein (FP)

with high affinity. As a result, nascent transcripts recruit tens to hundreds of MCP-FP or PCP-FP proteins to the active transcription loci, revealing fluorescent foci. This technique allows visualization of nascent transcripts in living cells with a high temporal resolution of a few seconds as well as in a single-cell resolution [62]. Initially developed in yeast, the MS2 and PP7-based live imaging technique has been implemented in many tissue types across organisms, including *Drosophila* and mouse embryos, mESCs, and other mammalian culture cells [63-66].

A combination of biosensors to visualize epigenetic states with the MS2/PP7 system to visualize nascent transcripts in living cells is poised to be a powerful tool to elucidate the timescale of epigenetic modification and the effect on gene expression. Expanding such imaging tools along with the dCas9-mediated epigenetic modifications will allow the analysis of epigenetic dynamics at specific loci with high spatial and temporal resolutions. With the ease of whole-genome sequencing, extensive amounts of data are available to compare the epigenetic states between the normal and diseased tissues. Such studies revealed that many epigenetic markers, including DNA methylation, histone modifications, and chromatin accessibility, are differentially expressed in the diseased tissues. Advancement in the field of epigenetic regulation will provide insights into new therapeutic approaches for various diseases associated with epigenetic modifications.

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REFERENCES

1. Z. E. Stine, Z. E. Walton, B. J. Altman, A. L. Hsieh and C. V. Dang, *Cancer Discov.*, **5**, 1024 (2015).
2. C. J. E. Ingram, C. A. Mulcare, Y. Itan, M. G. Thomas and D. M. Swallow, *Hum. Genet.*, **124**, 579 (2009).
3. A. Portela and M. Esteller, *Nat. Biotechnol.*, **28**, 1057 (2010).
4. J. Hunter, O. Rivero-Arias, A. Angelov, E. Kim, I. Fotheringham and J. Leal, *Am. J. Med. Genet. Part A*, **164**, 1648 (2014).
5. D. Colak, N. Zaninovic, M. S. Cohen, Z. Rosenwaks, W.-Y. Yang, J. Gerhardt, M. D. Disney and S. R. Jaffrey, *Science*, **343**, 1002 (2014).
6. T. Halevy, C. Czech and N. Benvenisty, *Stem Cell Reports*, **4**, 37 (2015).
7. X. S. Liu, H. Wu, M. Krzisch, X. Wu, J. Graef, J. Muffat, D. Hnisz, C. H. Li, B. Yuan, C. Xu, Y. Li, D. Vershkov, A. Cacace, R. A. Young and R. Jaenisch, *Cell*, **172**, 979 (2018).
8. D. Kumari and K. Usdin, *Hum. Mol. Genet.*, **19**, 4634 (2010).
9. M. Avitzour, H. Mor-Shaked, S. Yanovsky-Dagan, S. Aharoni, G. Altarescu, P. Renbaum, T. Eldar-Geva, O. Schonberger, E. Levy-Lahad, S. Epsztejn-Litman and R. Eiges, *Stem Cell Reports*, **3**, 699 (2014).
10. S. D. Sheridan, K. M. Theriault, S. A. Reis, F. Zhou, J. M. Madison, L. Daheron, J. F. Loring and S. J. Haggarty, *PLoS One*, **6**, e26203 (2011).
11. R. E. Amir, I. B. Van den Veyver, M. Wan, C. Q. Tran, U. Francke and H. Y. Zoghbi, *Nat. Genet.*, **23**, 185 (1999).
12. A. Martínez de Paz, L. Khajavi, H. Martin, R. Claveria-Gimeno, S. Tom Dieck, M. S. Cheema, J. V. Sanchez-Mut, M. M. Moksa, A. Carles, N. I. Brodie, T. I. Sheikh, M. E. Freeman, E. V. Petrotchenko, C. H. Borchers, E. M. Schuman, M. Zytnicki, A. Velazquez-Campoy, O. Abian, M. Hirst, M. Esteller, J. B. Vincent, C. E. Malnou and J. Ausió, *Epigenetics Chromatin*, **12**, 63 (2019).
13. M. Altuna, A. Urdánoz-Casado, J. Sánchez-Ruiz de Gordoa, M. V. Zelaya, A. Labarga, J. M. J. Lepesant, M. Roldán, I. Blanco-Luquin, Á. Perdones, R. Larumbe, I. Jericó, C. Echavarri, I. Méndez-López, L. Di Stefano and M. Mendioroz, *Clin. Epigenetics*, **11**, 91 (2019).
14. W. Sun, L. Zang, Q. Shu and X. Li, *Genomics*, **104**, 347 (2014).
15. R. L. Siegel, K. D. Miller, H. E. Fuchs and A. Jemal, *CA. Cancer J. Clin.*, **71**, 7 (2021).
16. P. Paydar, G. Asadikaram, H. Z. Nejad, H. Akbari, M. Abolhassani, V. Moazed, M. H. Nematollahi, G. Ebrahimi and H. Fallah, *J. Cell. Biochem.*, **120**, 13726 (2019).
17. B. W. Futscher, M. M. Oshiro, R. J. Wozniak, N. Holtan, C. L. Hanigan, H. Duan and F. E. Domann, *Nat. Genet.*, **31**, 175 (2002).
18. B. W. Futscher, M. M. O'Meara, C. J. Kim, M. A. Rennels, D. Lu, L. M. Gruman, R. E. B. Seftor, M. J. C. Hendrix and F. E. Domann, *Neoplasia*, **6**, 380 (2004).
19. N. Maass, M. Biallek, F. Rösel, C. Schem, N. Ohike, M. Zhang, W. Jonat and K. Nagasaki, *Biochem. Biophys. Res. Commun.*, **297**, 125 (2002).
20. S. A. Narod, BRCA mutations in the management of breast cancer: The state of the art (2010).
21. D. Glodzik, A. Bosch, J. Hartman, M. Aine, J. Vallon-Christersson, C. Reuterswärd, A. Karlsson, S. Mitra, E. Niméus, K. Holm, J. Häkkinen, C. Hegardt, L. H. Saal, C. Larsson, M. Malmberg, L. Rydén, A. Ehinger, N. Loman, A. Kvist, H. Ehrencrona, S. Nik-Zainal, Å. Borg and J. Staaf, *Nat. Commun.*, **11**, 3747 (2020).
22. R. N. Batra, A. Lifshitz, A. T. Vidakovic, S.-F. Chin, A. Sati-Batra, S.-J. Sammut, E. Provenzano, H. R. Ali, A. Dariush, A. Bruna, L. Murphy, A. Purushotham, I. Ellis, A. Green, F. E. Garrett-Bakelman, C. Mason, A. Melnick, S. A. J. R. Aparicio, O. M. Rueda, A. Tanay and C. Caldas, *Nat. Commun.*, **12**, 5406 (2021).
23. G. C. Hon, R. D. Hawkins, O. L. Caballero, C. Lo, R. Lister, M. Pelizzola, A. Valsesia, Z. Ye, S. Kuan, L. E. Edsall, A. A. Camargo, B. J. Stevenson, J. R. Ecker, V. Bafna, R. L. Strausberg, A. J. Simpson and B. Ren, *Genome Res.*, **22**, 246 (2012).
24. W. Jin, Q.-Z. Li, Y. Liu and Y.-C. Zuo, *Genomics*, **112**, 853 (2020).
25. A. Chatterjee, E. J. Rodger and M. R. Eccles, *Semin. Cancer Biol.*, **51**, 149 (2018).
26. M. V. Nemtsova, D. V. Zaletaev, I. V. Bure, D. S. Mikhaylenko, E. B. Kuznetsova, E. A. Alekseeva, M. I. Beloukhova, A. A. Deviatkin, A. N. Lukashev and A. A. Zamyatnin, *Front. Genet.*, **10**, 1 (2019).
27. M. C. de Andres, E. Perez-Pampin, M. Calaza, F. J. Santaclara, I. Ortea, J. J. Gomez-Reino and A. Gonzalez, *Arthritis Res. Ther.*, **17**, 233 (2015).
28. K. Nakano, J. W. Whitaker, D. L. Boyle, W. Wang and G. S. Firestein, *Ann. Rheum. Dis.*, **72**, 110 (2013).
29. L. de la Rica, J. M. Urquiza, D. Gómez-Cabrero, A. B. M. M. K. Islam, N. López-Bigas, J. Tegnér, R. E. M. Toes and E. Ballestar, *J. Autoimmun.*, **41**, 6 (2013).

30. E. Karouzakis, M. Trenkmann, R. E. Gay, B. A. Michel, S. Gay and M. Neidhart, *J. Immunol.*, **193**, 4945 (2014).
31. M. Trenkmann, M. Brock, R. E. Gay, C. Kolling, R. Speich, B. A. Michel, S. Gay and L. C. Huber, *Ann. Rheum. Dis.*, **70**, 1482 (2011).
32. T. T. Wada, Y. Araki, K. Sato, Y. Aizaki, K. Yokota, Y. T. Kim, H. Oda, R. Kurokawa and T. Mimura, *Biochem. Biophys. Res. Commun.*, **444**, 682 (2014).
33. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna and E. Charpentier, *Science*, **337**, 816 (2012).
34. L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang, *Science*, **339**, 819 (2013).
35. G. J. Knott and J. A. Doudna, *Science*, **361**, 866 (2018).
36. L. S. Qi, M. H. Larson, L. A. Gilbert, J. A. Doudna, J. S. Weissman, A. P. Arkin and W. A. Lim, *Cell*, **152**, 1173 (2013).
37. S. Konermann, M. D. Brigham, A. E. Trevino, J. Joung, O. O. Abudayyeh, C. Barcena, P. D. Hsu, N. Habib, J. S. Gootenberg, H. Nishimasu, O. Nureki and F. Zhang, *Nature*, **517**, 583 (2015).
38. A. C. Komor, A. H. Badran and D. R. Liu, *Cell*, **168**, 20 (2017).
39. S. R. Choudhury, Y. Cui, K. Lubecka, B. Stefanska and J. Irudayaraj, *Oncotarget*, **7**, 46545 (2016).
40. S. Morita, H. Noguchi, T. Horii, K. Nakabayashi, M. Kimura, K. Okamura, A. Sakai, H. Nakashima, K. Hata, K. Nakashima and I. Hatada, *Nat. Biotechnol.*, **34**, 1060 (2016).
41. C. Hu, X. Liu, Y. Zeng, J. Liu and F. Wu, *Clin. Epigenetics*, **13**, 1 (2021).
42. A. K. Giri and T. Aittokallio, *Front. Pharmacol.*, **10**, 1 (2019).
43. D. M. Sapozhnikov and M. Szyf, *Nat. Commun.*, **12** (2021).
44. A. Vojta, P. Dobrinić, V. Tadić, L. Bočkor, P. Korać, B. Julg, M. Klasić and V. Zoldoš, *Nucleic Acids Res.*, **44**, 5615 (2016).
45. P. Stepper, G. Kungulovski, R. Z. Jurkowska, T. Chandra, F. Krueger, R. Reinhardt, W. Reik, A. Jeltsch and T. P. Jurkowski, *Nucleic Acids Res.*, **45**, 1703 (2017).
46. L. Bintu, J. Yong, Y. E. Antebi, K. McCue, Y. Kazuki, N. Uno, M. Oshimura and M. B. Elowitz, *Science*, **351**, 720 (2016).
47. P. Cheung and P. Lau, *Mol. Endocrinol.*, **19**, 563 (2005).
48. D. Cano-Rodriguez, R. A. F. Gjaltema, L. J. Jilderda, P. Jellema, J. Dokter-Fokkens, M. H. J. Ruiters and M. G. Rots, *Nat. Commun.*, **7**, 12284 (2016).
49. D. Y. Kwon, Y.-T. Zhao, J. M. Lamonica and Z. Zhou, *Nat. Commun.*, **8**, 15315 (2017).
50. P. Perez-Pinera, D. D. Kocak, C. M. Vockley, A. F. Adler, A. M. Kabadi, L. R. Polstein, P. I. Thakore, K. A. Glass, D. G. Ousterout, K. W. Leong, F. Guilak, G. E. Crawford, T. E. Reddy and C. A. Gersbach, *Nat. Methods*, **10**, 973 (2013).
51. I. B. Hilton, A. M. D'Ippolito, C. M. Vockley, P. I. Thakore, G. E. Crawford, T. E. Reddy and C. A. Gersbach, *Nat. Biotechnol.*, **33**, 510 (2015).
52. M. B. Lustberg and B. Ramaswamy, *Curr. Breast Cancer Rep.*, **3**, 34 (2011).
53. S. M. G. Braun, J. G. Kirkland, E. J. Chory, D. Husmann, J. P. Calarco and G. R. Crabtree, *Nat. Commun.*, **8**, 560 (2017).
54. F.-S. Liang, W. Q. Ho and G. R. Crabtree, *Sci. Signal.*, **4**, 1 (2011).
55. Y. Gao, X. Xiong, S. Wong, E. J. Charles, W. A. Lim and L. S. Qi, *Nat. Methods*, **13**, 1043 (2016).
56. C.-L. Lo, S. R. Choudhury, J. Irudayaraj and F. C. Zhou, *Sci. Rep.*, **7**, 42047 (2017).
57. M. J. Kennedy, R. M. Hughes, L. A. Peteya, J. W. Schwartz, M. D. Ehlers and C. L. Tucker, *Nat. Methods*, **7**, 973 (2010).
58. C. Lungu, S. Pinter, J. Broche, P. Rathert and A. Jeltsch, *Nat. Commun.*, **8**, 649 (2017).
59. Y. Hori, N. Otomura, A. Nishida, M. Nishiura, M. Umeno, I. Suetake and K. Kikuchi, *J. Am. Chem. Soc.*, **140**, 1686 (2018).
60. E. Bertrand, P. Chartrand, M. Schaefer, S. M. Shenoy, R. H. Singer and R. M. Long, *Mol. Cell*, **2**, 437 (1998).
61. D. R. Larson, D. Zenklusen, B. Wu, J. A. Chao and R. H. Singer, *Science*, **332**, 475 (2011).
62. B. Lim, *Curr. Opin. Biotechnol.*, **52**, 49 (2018).
63. S. H. Keller, S. G. Jena, Y. Yamazaki and B. Lim, *Proc. Natl. Acad. Sci.*, **117**, 15096 (2020).
64. S. Syed, H. Wilky, J. Raimundo, B. Lim and A. A. Amodeo, *Proc. Natl. Acad. Sci.*, **118**, e2010210118 (2021).
65. J. Rodriguez, G. Ren, C. R. Day, K. Zhao, C. C. Chow and D. R. Larson, *Cell*, **176**, 213 (2019).
66. J. M. Alexander, J. Guan, B. Li, L. Maliskova, M. Song, Y. Shen, B. Huang, S. Lomvardas and O. D. Weiner, *Elife*, **8**, 1 (2019).

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