

As 5mC enters a programmable era: charted avenues for cancer understanding and intervention

Qianchuan Shi¹, Pengpeng Liu¹, Jian-Kang Zhu^{1,2} ✉, and Suna Sun¹ ✉

¹Institute of Advanced Biotechnology, School of Medicine and SUSTech Homeostatic Medicine Institute (SHMI), Southern University of Science and Technology, Shenzhen, 518055, China

²Faculty of Medicine, Macau University of Science and Technology, Macau, 999078, China

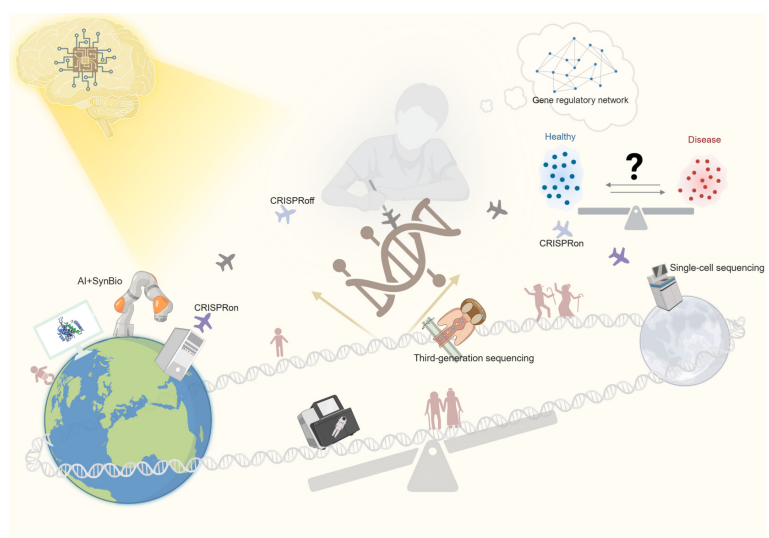


Cite This: *Oral Sci Homeost Med*, 2026, 2, 9610044



Read Online

ABSTRACT: Cancer is a loss of cellular homeostasis where epigenetic dysregulation drives adaptation and resistance beyond what genetic mutations alone can explain. 5-methylcytosine (5mC) is uniquely positioned as a programmable regulatory layer: it is writable, erasable and can be maintained through cell division, enabling persistent transcriptional control without DNA sequence change. While hypomethylating drugs (e.g., azacitidine or decitabine) are clinically established in multiple malignancies, their non-specific, genome-wide mode of action can lead to significant off-target effects and adverse events. CRISPR-based epigenome editors overcome this limitation by enabling locus-specific writing/erasing of 5mC; “hit-and-run” systems such as CRISPRoff further demonstrate that transient delivery can produce durable, heritable silencing. This Review explores emerging therapeutic directions: durable transcriptional suppression of disease-driving genes, reversal of therapy resistance, and immune-cell engineering. We further discuss how artificial intelligence can accelerate progress in editor design, multi-omics data integration, and delivery optimization—ushering in a transition from descriptive epigenetics to testable, mechanism-informed interventions.



KEYWORDS: DNA methylation (5mC), epigenome editing, precision oncology, homeostasis

1 Introduction

Cancer can be viewed as a failure of cellular homeostasis in which malignant cells continuously adapt to changing constraints^[1]. Disease progression occurs under shifting selective pressures exerted by therapy, immune surveillance, and microenvironmental conditions. These pressures concurrently drive clonal selection and reversible cell-state transitions, thereby broadening intratumoral heterogeneity and fostering treatment tolerance and eventual relapse. Consequently, elucidating the regulatory layers that determine the stability or plasticity of these cellular states becomes a cornerstone for developing mechanism-informed interventions^[2,3].

DNA methylation is one such regulatory layer. It is relatively stable and can help maintain gene-expression states over time and, in some contexts, across cell divisions. A hallmark of cancer methylomes is the coexistence of global hypomethylation and focal hypermethylation, the latter often concentrated at promoter-associated CpG islands. Global hypomethylation is associated with derepression of repetitive elements and genomic instability, whereas focal promoter hypermethylation can silence tumor suppressor genes and reinforce malignant transcriptional programs. Together, these features position 5mC as both a clinically actionable biomarker and a mechanistically testable regulatory variable, motivating site-specific perturbation strategies to establish causality at defined loci^[4-6].

Clinically, DNA methyltransferase inhibitors (DNMTis) such as azacitidine and decitabine have demonstrated efficacy in certain hematological malignancies, but they act genome-wide and lack locus specificity^[7]. This limits both therapeutic precision and mechanistic interpretation. Programmable epigenome editors

Received: March 12, 2026; **Revised:** March 24, 2026

Accepted: March 25, 2026

✉ Address correspondence to Jian-Kang Zhu, zhujk@sustech.edu.cn; Suna Sun, sunsn@sustech.edu.cn



address this gap: CRISPR–dCas platforms fused to methylation modifiers (e.g., DNMT3A for targeted methylation or TET1 for targeted demethylation) enable locus-specific rewriting of 5mC without altering DNA sequence^[8,9]. These tools allow direct perturbation of candidate regulatory elements to test whether site-specific methylation changes contribute to malignant phenotypes^[10].

Advances in artificial intelligence are increasingly reshaping how we interrogate and manipulate cancer methylomes. Machine learning models trained on multi-omic datasets can now identify methylation patterns associated with therapeutic resistance and prioritize candidate regulatory elements for experimental testing. In parallel, computational design frameworks are being applied to engineer more compact and specific epigenome editors, while deep learning approaches are beginning to decode how DNA methylation integrates with chromatin architecture and transcription-factor networks to control cell state. In this review, we examine three interconnected areas: first, how 5mC dysregulation contributes to cancer evolution and therapeutic resistance; second, how programmable methylation editors have matured from transient perturbations to tools capable of durable, locus-specific rewriting; and third, how computational approaches are guiding—and will increasingly enable—the rational design of targeted epigenetic interventions. Together, these developments suggest that the integration of AI-driven prediction with site-specific editing may provide a systematic framework to dissect, and ultimately reprogram, malignant cell states.

2 5mC in cancer: from biological mechanisms to clinical applications

DNA 5-methylcytosine (5mC) dysregulation is now recognized as a

central axis of cancer biology, governing genomic stability, tumor suppressor silencing, oncogene activation, and three-dimensional chromatin organization. These mechanistic insights have driven the development of both diagnostic biomarkers and therapeutic agents targeting the cancer methylome^[7,11–13].

2.1 Mechanisms of 5mC dysregulation in tumorigenesis

The genomic distribution of 5mC is governed by the balance between DNMT-mediated methylation and TET-initiated demethylation pathways. In tumors, this equilibrium is disrupted, yielding concurrent global hypomethylation and focal hypermethylation, particularly at promoter CpG islands (CGIs) (Fig. 1). Global hypomethylation promotes genomic instability by derepressing repetitive elements (e.g., LINE-1 and Alu elements) and can contribute to oncogenic transcriptional programs. Promoter hypomethylation has been associated with upregulation of oncogenes such as RAS, MYC, EGFR, and HER2, although their activation in cancer typically involves multiple converging mechanisms including gene amplification, translocation, and activating mutations. In contrast, focal CGI hypermethylation silences tumor suppressor genes (e.g., BRCA1, MLH1, VHL, CDKN2A)^[7,11,14].

Beyond direct effects on gene promoters, aberrant 5mC can reshape three-dimensional chromatin organization by compromising CTCF insulator function, enabling TAD boundary dissolution and enhancer hijacking—as documented in IDH-mutant gliomas (PDGFRA activation) and SDH-deficient gastrointestinal stromal tumors (FGF3 activation)^[15–18].

Collectively, these mechanisms position 5mC dysregulation as a central driver of tumorigenesis, motivating both diagnostic and therapeutic strategies targeting the cancer methylome.

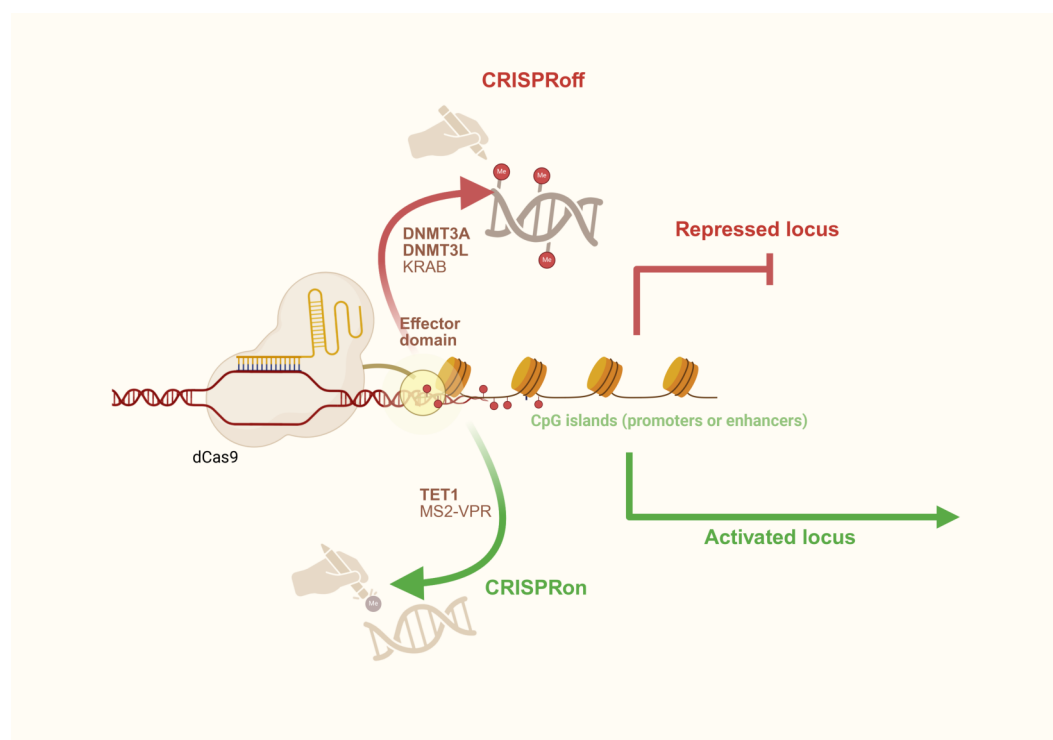


Figure 1 Multidimensional regulatory mechanisms and clinical translation of 5mC in cancer. 5mC methylation is dynamically regulated by DNMTs and TETs, with its dysregulation—characterized by global hypomethylation and focal hypermethylation—serving as a pivotal biomarker and therapeutic target. Figure was generated using BioRender.com.

2.2 Clinical translation of 5mC-targeted strategies

5mC methylation biomarkers exhibit high stability in cfDNA and are independent of tumor-specific mutational profiles, making them suitable for pan-cancer early screening and minimal residual disease detection (Fig. 1). Machine learning-based methylation classifiers enable precise tissue-of-origin determination, providing diagnostic solutions for cancer of unknown primary (CUP)^[19].

Given the pivotal role of 5mC in tumorigenesis, epigenetic drugs targeting 5mC regulatory machinery have been developed for clinical application. Among these agents, DNA methyltransferase inhibitors (DNMTis) (Fig. 1), such as azacitidine and decitabine, have demonstrated significant efficacy in hematological malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)^[14,20]. Importantly, 5mC-targeted therapies provide intervention strategies independent of DNA sequence mutations, thereby opening new avenues for overcoming clinical challenges such as drug resistance and tumor heterogeneity^[21].

Notably, Zhang et al. used single-cell transcriptomics and bulk epigenetic profiling to show that low-dose decitabine reverses DNA methylation-mediated immune gene silencing, restoring antitumor immunity within the tumor microenvironment^[22]. This potential "cold tumor" to "hot tumor" transformation has been validated in multiple independent studies, suggesting that 5mC regulation exerts dual effects: direct tumor cell modulation and tumor-immune network reprogramming^[18,21,22].

However, the non-specific effects of broad-spectrum methylation-modulating drugs have raised safety concerns, with some studies reporting potential carcinogenic risks and secondary malignancy occurrence^[23,24]. This challenge has driven the development of programmable tools for precise, site-specific regulation of 5mC modifications.

3 Evolution of editing tools: from transient to heritable

Although zinc finger proteins (ZFPs) and transcription activator-like effectors (TALEs) pioneered the field of epigenome editing, their practical application was hindered by complex design requirements, high costs, and limited scalability^[25,26]. The CRISPR-Cas platform transformed this landscape, offering modular, sgRNA-guided targeting with unprecedented accessibility. Fusion of catalytically dead Cas (dCas) effectors with epigenetic modifiers subsequently enabled rapid diversification of the epigenome editing toolkit^[27,30].

3.1 Programmable 5mC: capabilities and constraints

In 2016, Liu et al. and Vojta et al. reported the first modular CRISPR-based DNA methylation editors, fusing DNMT3A or TET1 catalytic domains directly to dCas9 for site-specific 5mC modification^[8,31]. This direct fusion design demonstrated the programmability of mammalian DNA methylation states^[31]. However, the induced effects were frequently transient; even when persistence was observed at specific loci or cell types, it was neither predictable nor reliably reproducible across cell divisions^[32,33].

This "epigenetic rebound" phenomenon exposed a critical bottleneck driven by converging molecular barriers. In early direct fusion designs, dCas9-DNMT3A-induced methylation concentrated within a relatively narrow window around the target site, insufficient to saturate CpG islands for stable silencing^[31].

Concurrently, pre-existing active histone marks (H3K4me3, H3K27ac) antagonized DNMT3A activity, while endogenous TET hydroxylases efficiently erased residual 5mC upon editor withdrawal, underscoring that single-modality interventions were inadequate for establishing heritable epigenetic memory^[33].

3.2 Evolution toward heritable memory: the "hit-and-run" strategy

To overcome these limitations, the field shifted toward combinatorial editing strategies. Amabile et al. provided early proof-of-concept by combining KRAB, DNMT3A, and DNMT3L as separate effector modules to achieve durable silencing^[32]. Núñez et al. refined this concept into CRISPRoff—a single-molecule fusion protein (dCas9-KRAB-DNMT3A-DNMT3L) that achieved durable, heritable silencing through a single transfection event (Fig. 2). Transient transfection alone established durable silencing in HEK293T cells, maintained in 38 of 39 single-cell clones after ~450 cell divisions (~15 months) without expression rebound. Critically, CRISPRoff overcame CpG island dependence, effectively silencing ~80% of human genes—including CGI-deficient loci (DYNC2LI1, LAMP2)—vastly expanding targeting scope^[10].

The corresponding CRISPRon system demonstrated precise reversibility. By co-expressing TET1 catalytic domain with multi-component activators (VPR or p65-Rta), CRISPRon robustly reactivated target genes within two days, achieving stable reactivation in more than 70% of cells. This "write-erase" control provided a versatile platform for dissecting causal gene-function relationships^[10].

With the write-erase toolkit thus established, the primary remaining challenge shifted from achieving durable silencing to enabling efficient in vivo delivery. The substantial coding size of dCas9 (often >4 kb) severely limits the packaging capacity of AAV vectors and the efficiency of non-viral delivery systems. Consequently, miniaturizing the editor while preserving its potency became a critical priority. Xu et al. replaced dSpCas9 with the compact dSF01 (Cas12i3 variant), achieving comparable efficiency with enhanced specificity and reduced off-target methylation. Because heritable 5mC editing requires only transient editor expression—the installed methylation being maintained through endogenous DNMT1-mediated copying during DNA replication—mRNA-LNP delivery is ideally suited to the hit-and-run editing paradigm. A single mRNA-LNP injection of the optimized editor (OFF-EE V2) silenced hepatic Pcsk9 in mice, reducing circulating PCSK9 by ~83% with a concomitant ~51% reduction in LDL cholesterol, sustained for at least 180 days^[30]. This result demonstrates that compact Cas variants paired with non-viral delivery can achieve clinical-grade, durable epigenetic silencing. The applicability of such compact, non-viral epigenetic editing platforms to oncology contexts is discussed in Sections 4 and 5.

Despite context-dependent variability in efficacy across cell lines and genomic loci, the field has now achieved the core capability for stable, heritable 5mC writing and erasure. The field has thus achieved the core capability for stable, heritable 5mC writing, enabling the first wave of programmable epigenetic silencing therapies to enter clinical evaluation across multiple disease contexts (Table 1).

4 Precision 5mC editing in oncology: therapeutic interventions and target discovery

Precise 5mC writing and erasure enables targeting of traditionally

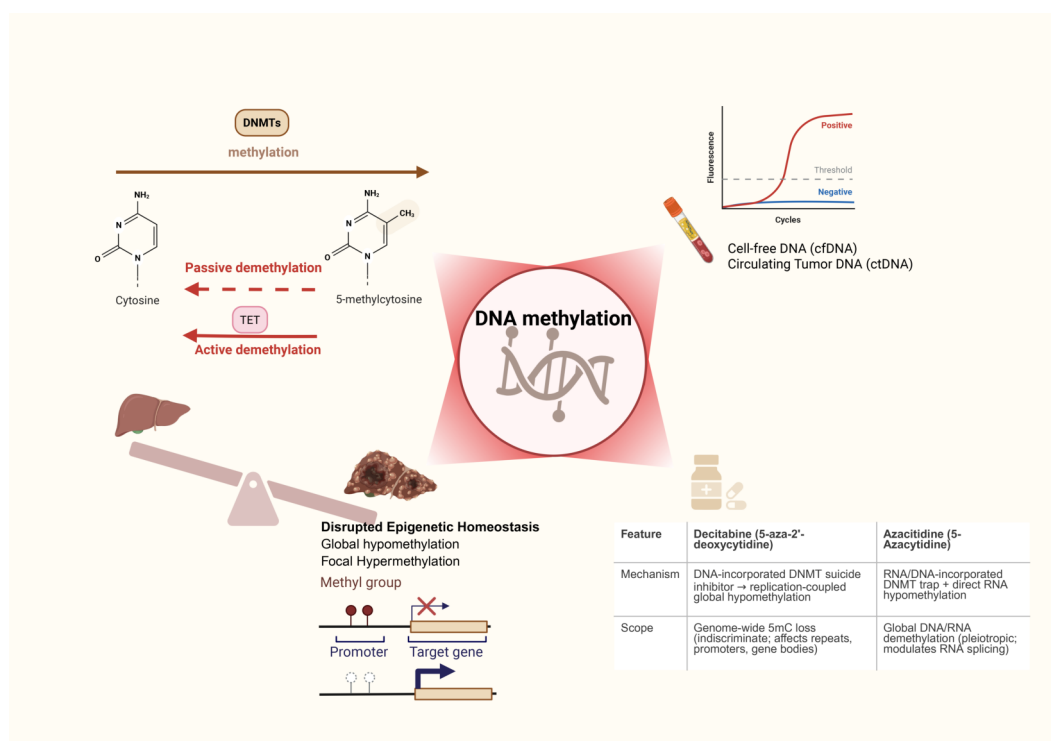


Figure 2 Programmable "write-erase" control of DNA methylation. CRISPRoff establishes heritable gene silencing by combining dCas9 with DNMT3A/L and KRAB domains to induce de novo methylation and heterochromatin assembly. Conversely, CRISPRon achieves precise reversibility through TET1-mediated demethylation and transcriptional activation via VPR/p65-Rta complexes. This integrated system enables the establishment and erasure of durable epigenetic memory across diverse genomic loci. Figure was generated using BioRender.com.

"undruggable" sites inaccessible to conventional pharmacological agents. By establishing durable epigenetic memory at targeted loci, these tools enable sustained modulation of gene expression programs with therapeutic relevance in oncology. Emerging evidence demonstrates substantial clinical translational potential (Table 2).

4.1 Targeted methylation

For "undruggable" targets lacking enzymatic pockets, such as the transcription factor MYC, methylation writing tools—including CRISPRoff and programmable epigenomic mRNA therapeutics—provide durable silencing of oncogenic drivers, as demonstrated in hepatocellular carcinoma models where MYC suppression inhibits tumor growth and enhances sensitivity to tyrosine kinase and immune checkpoint inhibitors^[29]. Similarly, this strategy holds particular clinical value in overcoming chemoresistance in glioblastoma (GBM), where MGMT is the key enzyme responsible for temozolomide resistance. CRISPRoff has enabled durable transcriptional silencing of MGMT, restoring chemosensitivity in GBM models^[34,35].

The sequence-level specificity of CRISPR epigenetic editing also enables allele-specific targeting. Kouroukli et al. developed an allele-specific epigenetic editing (ASEE) strategy that exploits single-nucleotide differences between mutant and wild-type TERT promoter sequences to selectively silence oncogenic alleles while preserving normal allele function. This genomic precision may enable therapeutic windows difficult to achieve with conventional approaches^[36].

Beyond oncogene silencing, targeted methylation writing can also create therapeutic vulnerabilities by disabling DNA repair pathways. He et al. showed that CRISPRoff-mediated silencing of

the BRCA1 promoter reduces homologous recombination repair capacity, thereby enhancing PARP inhibitor and cisplatin sensitivity in ovarian cancer cell models^[37]—illustrating how epigenetic silencing of a tumor suppressor can be strategically exploited to induce synthetic lethality.

4.2 Targeted demethylation

Targeted demethylation can reverse acquired drug resistance and suppress tumor progression by reactivating epigenetically silenced genes. For instance, hypermethylation of the NNT gene was found to be associated with cisplatin resistance in lung cancer by Xu et al., and chemosensitivity was restored through targeted demethylation of this locus, which suppressed protective autophagy flux^[38]. In the context of metastasis inhibition, targeted demethylation of miR-200c in breast cancer was performed by Zahraei et al., resulting in successful downregulation of downstream ZEB1 and KRAS, thereby reversing the epithelial-mesenchymal transition (EMT) process^[39]. Similarly, in cervical cancer, reactivation of EphA7 through demethylation was shown to exert significant tumor-suppressive functions via inhibition of the PI3K/AKT signaling pathway^[40].

BRCA1 promoter hypermethylation, which compromises homologous recombination repair, represents a key oncogenic driver in breast and ovarian cancers. Choudhury et al. first demonstrated that dCas9-TET1-mediated demethylation can reactivate BRCA1 expression, restoring its tumor-suppressive function^[41].

4.3 Enhancement of adoptive T cell therapy

Epigenetic editing offers a non-genotoxic alternative to nuclease-

Table 1 Clinical-stage and planned precision epigenetic editing therapeutics: oncology-directed programs and cross-disease platform validation studies. Non-oncology entries are included to illustrate delivery platform maturity and regulatory precedent relevant to oncological applications. All entries represent inhibitory epigenetic editing; activating approaches (e.g., dCas9-TET1 for tumor suppressor reactivation) remain preclinical. Phase designations follow ClinicalTrials.gov registration; information verified March 2026.

Company	Candidate	Platform Description	Delivery System	Target	Disease Indication	Trial ID	Phase	Status
nChroma Bio	CRMA-1001	Proprietary dCas9-based epigenetic silencer; targeted DNA methylation at HBV cccDNA and intDNA	LNP-mRNA (liver-directed)	HBV cccDNA and intDNA	Chronic hepatitis B	NCT07200193	1/2	Active, recruiting
Tune Therapeutics	TUNE-401	TEMPO platform: dCas9 + methyltransferase + epigenetic repressor; combined DNA methylation and transcriptional silencing at HBV cccDNA	LNP-mRNA (liver-directed)	HBV cccDNA and intDNA	Chronic hepatitis B	NCT06671093	1b	Active, recruiting
Epicrispr Biotechnologies	EPI-321	dCasONYX fused to DNA methyltransferase; targeted re-methylation of D4Z4 repeat region	AAVrh74 (muscle-tropic)	D4Z4 repeat region (chr 4q35)	Facioscapulohumeral muscular dystrophy (FSHD)	NCT06907875	1/2	Active, recruiting
Sangamo Therapeutics	ST-503	ZF-DBD + KRAB; H3K9me3-mediated transcriptional silencing without DNA methylation	AAV (STAC-BBB, CNS-penetrating)	Nav1.7 (SCN9A) promoter	Refractory peripheral neuropathy (SFN)	NCT06980948	1/2	Active, recruiting
Omega Therapeutics	OTX-2002	mRNA-encoded zinc finger + KRAB + DNMT; combined MYC promoter methylation and H3K9me3	LNP-mRNA (liver-directed)	MYC oncogene promoter	Hepatocellular carcinoma (HCC)	NCT05497453	1/2	Terminated
Scribe Therapeutics	STX-1150	ELXR platform: CasX-derived dCas fused to epigenetic effector domains; targeted 5mC writing and transcriptional silencing	LNP-mRNA (liver-directed)	PCSK9 promoter	Hypercholesterolemia/ASCVD	Not yet assigned	1 (planned)	IND-enabled; Phase 1 planned mid-2026

based gene disruption in ex vivo engineering of adoptive T cell therapy (ACT). Goudy et al. developed an all-RNA CRISPRoff platform that achieves durable silencing of therapeutically relevant genes, including FAS and PDCD1, in primary human T cells. Unlike CRISPR-Cas9 knockout, CRISPRoff-mediated epigenetic silencing avoids genomic cleavage, eliminating risks of chromosomal deletions or translocations associated with multiplexed nuclease editing. Notably, this silencing persists through multiple rounds of T cell stimulation and in vivo adoptive transfer, establishing a safer and scalable platform for next-generation CAR-T cell engineering^[42].

4.4 High-throughput screening and target discovery

Combined with sgRNA library technology, 5mC editing tools have emerged as powerful engines for establishing causal epigenetic relationships. While genome-wide methylation profiling can identify candidate loci associated with tumorigenesis, functional validation requires direct perturbation. CRISPR-based epigenetic screening addresses this gap: using dCas9-TET1 coupled with a customized sgRNA library, Tejedor et al. performed functional demethylation screening in colorectal cancer, identifying RSPO2 as a critical tumor suppressor among dozens of candidate loci^[43].

Beyond target discovery, screening studies have illuminated regulatory mechanisms. Notably, demethylation alone is often insufficient to activate deeply silenced genes; co-delivery of transcriptional activators such as VPR is required, revealing that DNA methylation and higher-order transcriptional repression constitute functionally distinct regulatory layers^[44].

5 Translational bottlenecks: a reality check

CRISPR-based epigenetic editing introduces a fundamentally different therapeutic modality for cancer: locus-specific regulatory control with an improved safety profile relative to nuclease-active gene editing. Unlike nuclease-active Cas9 systems, which introduce DNA double-strand breaks (DSBs), catalytically inactive Cas (dCas) proteins fused with epigenetic modifiers operate without creating permanent genetic lesions, thereby substantially reducing genotoxicity risks^[45]. However, biological and engineering challenges must be addressed before this technology can reach clinical application. These include optimizing delivery systems, ensuring editing specificity and regulatory safety, and achieving precision targeting within heterogeneous tumors.

5.1 The delivery challenge: vector optimization and tumor penetration

Effective delivery of CRISPR-based epigenetic editing systems to solid tumors requires overcoming substantial physiological and immunological barriers. The tumor microenvironment (TME) poses multiple physical barriers, including dense extracellular matrix (ECM) deposition, elevated interstitial fluid pressure (IFP)—which can reach ~40 mmHg in some cancers (versus ~3 to +3 mmHg in normal tissues)—and structurally abnormal vasculature that creates hypoxic, poorly perfused regions^[46]. These physical forces impede therapeutic agent transport while fueling tumor progression and treatment resistance through hypoxia-induced stemness and immune evasion. Concurrently, pre-existing

Table 2 Representative programmable DNA methylation editing studies in cancer research and immune cell engineering (2016–2025). Representative studies illustrate the methodological breadth and cancer-relevant applications of programmable 5mC editing, spanning established cancer cell lines, patient-derived xenograft models, hematopoietic progenitor cells, and primary T cell engineering for cancer immunotherapy. Entries are ordered chronologically. For mechanistic details, readers are referred to primary literature via the provided DOIs; information verified March 2026.

Year	First Author	Epigenetic Editing System	Editing Directionality	Target Gene/Locus	Cancer Type/Model System	In Vivo Validation	Journal, DOI
2016	Vojta A et al.	dCas9-DNMT3A	Writing	BACH2, IL6ST promoters	HEK293T	No	Nucleic Acids Res, 10.1093/nar/gkw159
2016	Choudhury SR et al.	dCas9-TET1	Erasure	BRCA1 promoter	Breast cancer (MCF7, HeLa)	No	Oncotarget, 10.18632/oncotarget.10234
2019	Wang Q et al.	dCas9-multiGCN4/scFv-TET1CD	Erasure	SARI promoter	Colon cancer (HCT116, RKO, SW480, HT29, HCT15)	Yes (xenograft)	Cancer Lett, 10.1016/j.canlet.2019.01.040
2021	Nuñez JK et al.	CRISPRoff (dCas9-KRAB-DNMT3A-DNMT3L)	Writing	Multiple loci	HEK293T, iPSCs (MDA-MB-231 for enhancer validation)	No	Cell, 10.1016/j.cell.2021.03.025
2023	Saunderson EA et al.	dCas9-DNMT3A-DNMT3L	Writing	p15 (CDKN2B)	Hematologic malignancy (primary human HSPCs)	Yes (HSPC engraftment, NSG mice)	PNAS, 10.1073/pnas.2300224120
2023	Kouroukli A et al.	SunTag-dCas9-DNMT3A/3L (low-affinity mutant; three-plasmid system)	Writing	Mutant TERT promoter	Hepatoblastoma (Hep-G2), lung adenocarcinoma (A549)	No	Clin Epigenetics, 10.1186/s13148-023-01599-2
2023	Tejedor JR et al.	dCas9-TET1	Erasure	56 CpG island promoters (incl. RSPO2)	Colorectal cancer (DLD1, HCT116)	No	Clin Epigenetics, 10.1186/s13148-023-01546-1
2024	He W et al.	Custom dCas9-based epigenetic editor	Writing	BRCA1 promoter (TSS-proximal CpG)	Ovarian cancer (A2780, SKOV3, OVCAR3)	No	Epigenetics, 10.1080/15592294.2024.2357518
2024	Senapedis W et al.	mRNA-encoded ZF-KRAB-DNMT3A-DNMT3L (OTX-2002)	Writing	MYC IGD regulatory elements	HCC (Hep 3B, SK-HEP-1)	Yes (subcutaneous + orthotopic xenograft)	Nat Commun, 10.1038/s41467-024-52202-y
2024	Weber R et al.	CRISPRoff (dCas9-KRAB-DNMT3A-DNMT3L)	Writing	MGMT promoter	Glioblastoma (T-325, LN-18)	No	Front Oncol, 10.3389/fonc.2024.1342114
2025	Bayraktar E et al.	Liposomal dCas9-TET1	Erasure	BARD1 promoter	Ovarian cancer (SKOV3ip1 intraperitoneal model)	Yes (ovarian xenograft)	Cell Rep Med, 10.1016/j.xcrm.2025.102329
2025	Zahraei M et al.	dCas9-TET1	Erasure	miR-200c promoter	Breast cancer (MCF7, MDA-MB-231)	No	Sci Rep, 10.1038/s41598-025-16911-8
2025	Zhang B et al.	dCas9-DNMT3L-DNMT3A	Writing	mrDEG promoters (CAVIN2, TENT5B, ARL4D, DUSP1, P3H2, MMP28)	Breast cancer progression (MCF10 series: normal → atypical hyperplasia → metastatic)	No	Cell Mol Life Sci, 10.1007/s00018-024-05562-z
2025	Lin K et al.	CRISPRoff (dCas9-KRAB-DNMT3A-DNMT3L)	Writing	MGMT promoter	Glioblastoma (LN18, T98G, patient-derived primary GBM)	Yes (orthotopic GBM xenograft)	Neuro Oncol, 10.1093/neuonc/noaf055
2025	Xu D et al.	CRISPRoff (dCas9-KRAB-DNMT3A-DNMT3L); RENDER eVLP delivery	Writing	Multiple genes (MAPT/Tau, endogenous T cell targets)	iPSC-derived neurons; primary human T cells (in vitro)	Yes (iPSC neuron in vivo silencing)	Nat Commun, 10.1038/s41467-025-63167-x
2025	Goudy L et al.	CRISPRoff/CRISPRon (mRNA delivery)	Writing/Erasure	Multiple genes (up to 5 simultaneous)	B-cell leukemia (Nalm6 primary model); solid tumour (auxiliary validation)	Yes (Nalm6 leukemia mouse model)	Nat Biotechnol, 10.1038/s41587-025-02856-w

antibodies against Cas proteins—particularly in individuals with prior exposure to bacterial pathogens—can trigger inflammatory responses and rapid clearance of therapeutic vectors^[47], complicating strategies for repeated dosing.

Viral vectors, particularly adeno-associated viruses (AAVs), have been extensively explored for CRISPR delivery due to their

established safety profiles and efficient nuclear entry. However, the packaging constraint of AAVs (~4.7 kb) limits the delivery of larger dCas-effector fusions, necessitating the use of smaller Cas orthologs such as *Campylobacter jejuni* Cas9 (CjCas9, ~3.0 kb) or split-intein systems^[47]. More recently, a new class of miniature nucleases, termed OMEGA systems (including IscB and TnpB), has emerged

as a promising alternative^[48,49]. Derived from IS200/IS605 transposon families as evolutionary ancestors of Cas9 and Cas12, these enzymes are approximately 350–500 amino acids in size—less than half the length of CjCas9—enabling single-AAV packaging of even complex dCas-effector fusions^[50]. Similarly, engineered Cas12f variants (e.g., enUn1Cas12f1, hpCasMINI) have shown markedly improved activity in mammalian cells and enable efficient in vivo base editing^[51,52]. These miniature systems thus offer a powerful platform to circumvent AAV packaging constraints and expand the applicability of CRISPR-based epigenome engineering.

As a non-viral alternative, lipid nanoparticles (LNPs) demonstrate significant promise, as exemplified by FDA-approved RNA therapeutics. Ionizable LNPs (iLNPs) enable pH-dependent endosomal escape and can achieve high-efficiency gene editing in vivo when optimized for specific cargo formats^[53]. Selective organ targeting (SORT) technology has expanded LNP tropism beyond the liver to include the lung and spleen by incorporating permanently cationic lipids such as DOTAP^[54]. For tumor targeting, surface modifications with ligands—including hyaluronan (targeting CD44), iRGD peptides, or tumor-associated antigen antibodies—enhance cancer cell uptake^[55]. Nevertheless, deep tumor penetration remains challenging, with nanoparticles often confined to perivascular regions. Strategies to normalize tumor vasculature or remodel the ECM, such as co-delivery of hyaluronidase, are being explored to improve intratumoral distribution.

For glioblastoma and other central nervous system malignancies, delivery is further complicated by the blood-brain barrier (BBB). Investigated strategies include focused ultrasound-mediated BBB disruption, receptor-mediated transcytosis, and direct intratumoral injection. Compact editors based on diverse Cas scaffolds—including dSF01^[56], CasMINI^[57], IscB^[58], and CHARM^[59]—are critical for CNS applications, as their smaller size improves BBB penetration and enables delivery via a single AAV vector.

Addressing immune responses requires multiple complementary strategies: transient expression formats (mRNA or RNP) to limit Cas exposure duration; smaller Cas orthologs from less common bacterial species to evade pre-existing immunity; surface humanization of Cas proteins to reduce antigen presentation; and non-immunogenic vehicles such as engineered extracellular vesicles^[60]. Engineered virus-like particles (eVLPs) represent an emerging modality that combines the efficient cellular entry of viral particles with the transient, integration-free profile of RNP delivery. The RENDER platform demonstrated that CRISPRoff-loaded eVLPs achieve robust epigenetic silencing across multiple human cell types, including primary T cells^[61], demonstrating compatibility with the hit-and-run epigenetic editing paradigm.

5.2 Safety considerations: from off-target binding to regulatory cascade effects

While the absence of DSBs substantially reduces genotoxicity (Section 5 introduction), several layers of safety concern remain specific to epigenetic editors. Off-target binding of dCas fusion proteins may induce unintended transcriptional dysregulation at sites with partial sgRNA complementarity^[45]. Systematic studies demonstrate that off-target effects in CRISPR interference/activation (CRISPRi/a) screens can confound the identification of functional regulatory elements, particularly when targeting non-coding regions with limited sgRNA design flexibility^[62].

Engineered high-fidelity Cas9 variants have substantially

advanced specificity. SpCas9-HF1, harboring substitutions at non-specific DNA-contacting residues (N497A, R661A, Q695A, Q926A), achieves undetectable off-target cleavage at most sites while retaining on-target activity comparable to wild-type SpCas9 with the majority of sgRNAs tested in plasmid delivery format^[63]. Similarly, the R691A HiFi Cas9 mutant—identified through unbiased bacterial selection—maintains near-wild-type on-target efficiency when delivered as ribonucleoprotein (RNP) complexes while dramatically reducing off-target editing^[64]. These variants can be readily adapted for epigenetic editing by fusion with DNMT3A, TET1, or other effector domains.

A key safety consideration unique to epigenome editing is epigenetic off-target effects. Unlike nuclease-active Cas9 (assessed at the DNA sequence level), epigenetic editors can induce widespread methylation changes: dCas9-DNMT3A fusions deposit methylation at thousands of off-target CpG sites, especially at developmentally regulated gene promoters—many of which harbor bivalent chromatin states (co-marked by H3K4me3 and H3K27me3) that are particularly susceptible to de novo methylation—as revealed by WGBS and reduced representation bisulfite sequencing (RRBS)^[65]. This may lead to persistent silencing of tumor suppressors or oncogene activation, with carcinogenic risks requiring long-term studies in primary cells and animal models. Mitigation strategies include the use of high-fidelity dCas9 variants, optimized sgRNA algorithms, and split-activity editors (active only upon dCas-sgRNA complex formation)^[66].

Such split-activity designs restrict effector domain catalysis to sites where the complete dCas-sgRNA complex is stably bound, thereby structurally limiting methylation deposition at transiently contacted off-target loci—a safeguard that addresses epigenetic spreading but not the on-target regulatory consequences discussed below.

A distinct and underappreciated category of risk stems not from imprecise targeting but from the propagative nature of epigenetic modifications themselves. At the most local level, DNMT3A-mediated methylation can spread from targeted CpG sites to neighboring cytosines^[67], particularly in CpG-dense regions, potentially extending the silencing footprint beyond the intended regulatory element. At the chromatin level, as discussed in Section 2.1, aberrant methylation can compromise CTCF insulator function and reshape three-dimensional genome organization, enabling enhancer hijacking and oncogene activation—mechanisms now well documented in IDH-mutant gliomas^[17] and SDH-deficient tumors^[18]. The critical concern for therapeutic 5mC editing is that both off-target methylation deposition and on-target local spreading could, in principle, trigger similar 3D genomic restructuring at unintended loci. Because the relationship between a given methylation change and its downstream effects on chromatin architecture is highly context-dependent and not yet predictable from sequence alone, the full scope of these risks remains difficult to assess prior to experimental validation. Beyond insulator disruption, silencing a target promoter may release its associated enhancers to activate alternative genes within the same regulatory domain—a risk that scales with the regulatory reach of the targeted locus. Such considerations underscore the need for safety evaluation frameworks that assess not only editing specificity but also the regulatory cascade effects of targeted methylation changes.

In the tumor context, these regulatory risks acquire additional dimensions. Intratumoral epigenetic heterogeneity means that

identical edits may produce divergent outcomes across clonal subpopulations occupying different chromatin states^[3]—as illustrated by Vizoso et al., who showed that comparable increments of IGF2P2 promoter methylation produced opposing transcriptional outcomes across tumor cell lines with distinct baseline chromatin states^[60]—potentially promoting rather than restricting phenotypic plasticity and cell-state transitions. Although therapeutic 5mC editing has not yet been reported to cause unintended bystander effects on immune recognition, differentiation programs, or compensatory resistance pathways, the precedent set by methylation-driven regulatory rewiring in cancer (Section 2.1) suggests that such outcomes cannot be excluded, particularly when editing targets reside in regulatory-dense genomic regions. Because these potential consequences depend on the pre-existing chromatin state of each cell—which varies across subclones within a single tumor—they are inherently difficult to predict at the population level.

Addressing these multilayered safety concerns will benefit from computational tools capable of predicting the multimodal regulatory consequences of targeted methylation changes (Section 6.3). Even when these safety challenges are adequately managed, a further layer of complexity arises from tumor heterogeneity.

5.3 Heterogeneity and resistance: precision intervention and high-throughput screening

Even with optimized delivery and high-fidelity editing tools, therapeutic success ultimately depends on addressing tumor heterogeneity—a fundamental biological complexity that demands precision intervention strategies. Intratumoral heterogeneity (ITH) encompasses both genetic and epigenetic diversity, with the latter exhibiting greater dynamism and plasticity. Cancer cells can transition between distinct epigenetic states, facilitating the emergence of drug-tolerant subclones that drive treatment failure and disease relapse^[3]. Crucially, the reversible nature of epigenetic-mediated resistance suggests that combination approaches targeting both genetic and epigenetic drivers may achieve more durable responses^[60]—because epigenetic resistance states, unlike fixed genetic mutations, can in principle be reversed by targeted demethylation, enabling sequential or combinatorial intervention strategies.

While the reversibility of epigenetic modifications offers therapeutic opportunities, it simultaneously demands precise intervention strategies. For instance, hypermethylation of DNA repair genes can induce resistance to chemotherapy and radiation, whereas global hypomethylation induced by non-specific inhibitors may paradoxically exacerbate genomic instability^[7]. CRISPR-based epigenetic editing has the potential to overcome these limitations by enabling locus-specific methylation reprogramming. However, identifying optimal target loci requires comprehensive epigenomic profiling and functional validation.

Beyond the target discovery applications described in Section 4.4, high-throughput screening platforms serve a critical translational function: systematically mapping the regulatory landscape to identify optimal intervention targets and predict editing outcomes at scale. CRISPRi-based screens coupled with flow cytometry or single-cell RNA sequencing enable the systematic interrogation of cis-regulatory elements controlling oncogene expression^[70]. Technologies such as Spear-ATAC allow direct association of single-cell epigenetic profiles with sgRNA identity, dramatically increasing

throughput while reducing per-cell costs^[71]. These approaches can identify critical chromatin boundaries, enhancer elements, and promoter regions that maintain oncogenic transcription programs—knowledge essential for designing effective epigenetic therapies.

Integrating experimental screening data with patient-derived multi-omics profiles—methylation, transcriptomic, and chromatin accessibility—and computational prediction tools (Section 6.3) can identify targets most likely to be both epigenetically accessible and therapeutically consequential in a given tumor context. As these technologies advance toward clinical application, critical translational requirements will include not only scalable manufacturing and standardized quality metrics but also long-term monitoring strategies to assess the stability of engineered methylation states across successive cell divisions in vivo.

6 AI-driven approaches to programmable 5mC editing

The translational bottlenecks outlined above—editor size and immunogenicity, delivery to heterogeneous solid tumors, off-target activity, and the need for patient-specific target selection—each present optimization challenges that exceed the throughput of conventional experimental workflows. The programmable rewritability of 5mC—writable, erasable, and heritable—positions it uniquely among epigenetic marks for such AI-augmented intervention: what was once a passively observed modification is becoming a designable regulatory layer. Acting as a cognitive accelerator rather than a mere computational tool, AI now enables researchers to navigate the combinatorial complexity of editor design, target selection, and outcome prediction at a scale unattainable through experimental iteration alone. This extends to an emerging capacity to preview the regulatory consequences of a site-specific methylation edit before ink is committed to the biological page. This convergence is driving a transition from correlative epigenomics to rational, site-specific rewriting of cancer methylomes. Below we examine this acceleration along three axes: generative design of compact, specific editors (Section 6.1); base-resolution profiling of tumor methylome heterogeneity (Section 6.2); and computational prediction of editing outcomes (Section 6.3).

6.1 Generative AI and closed-loop optimization of epigenetic editors

The integration of robotic automation with generative AI is accelerating epigenetic tool development through closed-loop optimization cycles. Computational design generates candidate sequences, automated platforms execute high-throughput assembly and testing, and real-time feedback refines predictive models. This paradigm overcomes cognitive and throughput limitations of human-driven experimentation, enabling efficient exploration of sequence spaces beyond natural evolution or rational design.

Deep learning architectures such as OpenCRISPR-1^[72] and Evo^[73] exemplify this shift by decoding biomolecular patterns to generate editors with programmable functions or novel mechanisms. The successor model Evo 2^[74] extends this capability to eukaryotic genomes, demonstrating zero-shot prediction of clinically significant BRCA1 variant effects without task-specific fine-tuning—a result directly relevant to the BRCA1 promoter demethylation strategies discussed in Section 4.2. Protein evolution

frameworks such as MULTI-evolve^[75], which achieved 9.8-fold activity enhancement of dCasRx through ML-guided multi-mutant prediction trained on as few as ~200 strategically selected pairwise variants, demonstrate a readily transferable paradigm for optimizing epigenetic editor effector domains—such as DNMT3A catalytic variants and KRAB repressor modules—complementing general-purpose protein design platforms (e.g., ProteinMPNN^[76], RFdiffusion^[77]).

A demonstration of this approach is the LUMI-lab platform—a foundation model-driven self-driving laboratory that discovered novel ionizable lipids within 10 iterative cycles and achieved high CRISPR-Cas9 editing efficiency (20.3%) in mouse lung epithelium via inhaled LNP delivery^[78]. This closed-loop lipid optimization paradigm is directly transferable to tumor-targeted LNP development for in vivo epigenetic editing. Comparable preclinical advances include all-RNA multiplex epigenetic programming of primary human T cells^[42] and CRISPRoff-mediated chemosensitization in glioblastoma models^[34].

AI-driven structural optimization is addressing two key bottlenecks in epigenetic therapeutics: delivery constraints and long-term durability. Miniaturized editors such as CHARM^[59] alleviate AAV packaging limitations, while precision-designed systems including EvoETR^[79] and CRISPRoff^[10] enable “hit-and-run” modifications with heritable persistence, offering a path toward effective transient interventions. Notably, EvoETR exemplifies a complete AI-to-bedside loop: computationally designed epigenetic repressors achieved durable in vivo gene silencing through a single transient administration, demonstrating that AI-guided editor engineering can yield clinically relevant durability without permanent genomic modification^[79].

6.2 AI-Enhanced detection of tumor methylome heterogeneity at base resolution

Identifying optimal 5mC editing targets in heterogeneous tumors requires base-resolution measurement of epigenetic states across single cells. AI converts population-averaged readouts into single-molecule and single-cell representations, enabling reconstruction of epigenetic heterogeneity.

Standard bisulfite sequencing profiles 5mC but fragments DNA, precludes long-range phasing, and cannot distinguish 5mC from 5-hydroxymethylcytosine (5hmC). Nanopore sequencing with deep learning enables direct 5mC calling without chemical treatment. Transformer approaches like Rockfish^[80] enhance read-level detection across sequencing chemistries with benchmark improvements. Methods such as DeepBAM^[81] decode methylation from aligned data, showing bisulfite-seq concordance, though alignment-dependent.

Epigenetic regulation is inherently multi-layered, motivating integrative measurement beyond single-modality assays. Platforms such as scDEEP-mC^[77] enable high-coverage, allele-resolved single-cell methylomes, while sciMETv3^[82] increases throughput via combinatorial indexing for lineage-aware tumor studies. Emerging multi-omic platforms^[83] further couple methylation with transcriptional state and inferred copy-number variation, providing integrated views of epigenetic and transcriptional heterogeneity. Multi-modal transformers such as EpiBERT^[84] integrate DNA sequence with chromatin accessibility, improving regulatory prediction and generalization to unseen genomic contexts—advancing unified interpretation of epigenetic landscapes.

Together, these advances provide the data foundation for selecting and validating 5mC editing targets within highly heterogeneous tumor microenvironments.

6.3 Predicting downstream consequences of site-specific methylation editing

A critical gap in therapeutic 5mC editing is the ability to predict the downstream regulatory consequences of site-specific methylation changes within a given tumor context—consequences spanning chromatin remodeling, transcription factor occupancy shifts, and gene expression changes that remain difficult to anticipate from editing coordinates alone. Computational models are beginning to address this gap from complementary angles.

At the methylation-specific level, DiffuCPG^[85] employs diffusion-based generative modeling to address extreme sparsity in single-cell methylation data, enabling genome-wide methylome imputation with capacity to incorporate long-range chromatin interaction signals (e.g., Hi-C)—providing a more complete substrate for identifying candidate 5mC editing targets. In a complementary approach, Evo 2^[74] has demonstrated generative epigenomics capabilities, including modeling how designed DNA sequences achieve specified chromatin accessibility profiles, suggesting potential for in silico prediction of regulatory outcomes relevant to 5mC editing. Bridging methylation state to transcriptional output, DeepMethyGene^[86] provides a direct predictive link from CpG methylation patterns to gene expression levels, offering a computationally tractable way to estimate transcriptional consequences of targeted methylation changes before experimental validation.

While the models above operate at the methylation level, understanding the full regulatory cascade of a 5mC edit requires broader sequence-to-function prediction. AlphaGenome^[87], successor to Enformer^[88], takes 1 Mb of DNA sequence as input and predicts thousands of functional genomic tracks—including chromatin accessibility, histone modifications, transcription factor binding, and three-dimensional chromatin contacts—at single-base-pair resolution. This multimodal scope is particularly relevant to 5mC editing safety assessment, enabling systematic in silico evaluation of on-target chromatin consequences prior to experimental validation. In a cancer-relevant demonstration, AlphaGenome accurately recapitulated regulatory mechanisms of non-coding mutations upstream of the TAL1 oncogene in T-ALL.

Looking further ahead, virtual cell models represent an emerging, largely conceptual direction. Complementary frameworks address different levels of the perturbation-to-phenotype prediction chain: CellOracle^[89] infers gene regulatory networks to identify which transcription factors are most likely affected by a given perturbation, while GEARS^[83] directly predicts transcriptional outcomes, enabling prioritization of experimental targets. Both have demonstrated utility for transcription factor perturbations, though their adaptation to site-specific 5mC editing—a mechanistically distinct perturbation class—will require large-scale paired methylation-perturbation datasets not yet widely available. Foundation models for single-cell biology (e.g., Geneformer^[90], scGPT^[91]) could serve as computational substrates for integrating methylation, transcriptomic, and chromatin data within a single predictive framework. Integrating such platforms with methylation-specific predictors (DiffuCPG^[85], SCENIC+^[92], DeepMethyGene^[86]) suggests a path toward cascaded epigenetic-to-phenotype modeling, though realizing this will require matched

perturbation-response datasets and rigorous validation in primary tumor models.

7 Conclusion

Disruption of 5mC homeostasis is not confined to cancer, it was also observed across numerous diseases, including aging and neurodegenerative disorders such as Alzheimer's disease^[5,93]. Therapeutic epigenetic remodeling finds a primary proving ground in oncology, where durable transcriptional control holds the potential to complement or even surpass conventional treatments. The emerging ability to treat 5mC not only as a biomarker but as a programmable, reversible regulatory layer marks a pivotal shift: in principle, locus-specific epigenetic intervention can move beyond the global, non-specific effects associated with traditional hypomethylating agents and enable regulation of disease-driving genes that are difficult to modulate with small molecules alone^[10].

Despite rapid progress, clinical translation remains constrained by practical hurdles—including efficient non-viral delivery, robustness in heterogeneous tumor microenvironments, and stringent control of off-target activity and long-term safety. Concurrently, advances in epigenetics, tumor biology, protein engineering, and computational modeling, together with accelerating AI adoption, are driving multidisciplinary convergence. Rather than replacing established biological insight, AI is increasingly serving as an enabling layer: expanding design search spaces, supporting data-driven optimization, and motivating the development of “virtual cell” or digital-twin-like frameworks for hypothesis testing and intervention prioritization—while also highlighting the need for rigorous calibration and validation.

The convergence of high-resolution measurement, precision editing, and more scalable design and optimization workflows is making epigenetic reprogramming toward restored cellular homeostasis progressively more attainable. Realizing this opportunity will require sustained cross-disciplinary collaboration among biologists, computational scientists, clinicians, and engineers to build matched perturbation datasets, robust delivery solutions, and safety-first translation pathways.

Acknowledgement

The authors acknowledge and are grateful for the financial support provided by the Guangdong S&T Program (Grant No. [2024B1111130001]) and Shenzhen Science and Technology Program (Grant No. [KJZD20240903102703005]) to J.-K.Z. We thank BioRender (biorender.com) for providing the graphical tool used to create the schematic diagrams in this manuscript. Finally, we appreciate the valuable discussions and support from all members of the Jian-Kang Zhu laboratory.

Data available statement

N/A

Author contribution

S.S. and J.-K.Z. provided overall direction, secured funding, and supervised the development of the commentary. Q.S. and S.S. drafted the manuscript. Q.S. prepared the figure. P.L., J.-K.Z., and S.S. provided valuable insights and contributed to refining the manuscript.

Ethics approval and consent

N/A

Consent for publication

All authors have reviewed and approved the manuscript for publication.

Conflicts of interest

Author Jian-Kang Zhu is the Editor-in-Chief of this journal, but he is not involved in the peer review or decision of this article.

References

- [1] Feinberg, A. P.; Ohlsson, R.; Henikoff, S. The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* **2006**, *7*, 21–33.
- [2] Flavahan, W. A.; Gaskell, E.; Bernstein, B. E. Epigenetic plasticity and the hallmarks of cancer. *Science* **2017**, *357*, eaal2380.
- [3] Easwaran, H.; Tsai, H. C.; Baylin, S. B. Cancer epigenetics: Tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol. Cell* **2014**, *54*, 716–727.
- [4] Jones, P. A.; Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* **2002**, *3*, 415–428.
- [5] Feinberg, A. P.; Vogelstein, B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **1983**, *301*, 89–92.
- [6] Baylin, S. B.; Jones, P. A. A decade of exploring the cancer epigenome: Biological and translational implications. *Nat. Rev. Cancer* **2011**, *11*, 726–734.
- [7] Sadida, H. Q.; Abdulla, A.; Al Marzooqi, S.; et al. Epigenetic modifications: Key players in cancer heterogeneity and drug resistance. *Transl. Oncol.* **2024**, *39*, 101821.
- [8] Liu, X. S.; Wu, H.; Ji, X.; et al. Editing DNA methylation in the mammalian genome. *Cell* **2016**, *167*, 233–247.e17.
- [9] Morita, S.; Noguchi, H.; Horii, T.; et al. Targeted DNA demethylation *in vivo* using dCas9–peptide repeat and scFv–TET1 catalytic domain fusions. *Nat. Biotechnol.* **2016**, *34*, 1060–1065.
- [10] Nuñez, J. K.; Chen, J.; Pommier, G. C.; et al. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell* **2021**, *184*, 2503–2519.e17.
- [11] Nishiyama, A.; Nakanishi, M. Navigating the DNA methylation landscape of cancer. *Trends Genet.* **2021**, *37*, 1012–1027.
- [12] Hanahan, D. Hallmarks of cancer: New dimensions. *Cancer Discov.* **2022**, *12*, 31–46.
- [13] Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674.
- [14] Zhao, A. L.; Zhou, H.; Yang, J. R.; et al. Epigenetic regulation in hematopoiesis and its implications in the targeted therapy of hematologic malignancies. *Signal Transduct. Target. Ther.* **2023**, *8*, 71.
- [15] Kim, K. L.; Rahme, G. J.; Goel, V. Y.; et al. Dissection of a CTCF topological boundary uncovers principles of enhancer-oncogene regulation. *Mol. Cell* **2024**, *84*, 1365–1376.e7.
- [16] Deng, S. W.; Feng, Y. L.; Pauklin, S. 3D chromatin architecture and transcription regulation in cancer. *J. Hematol. Oncol.* **2022**, *15*, 49.
- [17] Flavahan, W. A.; Drier, Y.; Liao, B. B.; et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* **2016**, *529*, 110–114.
- [18] Flavahan, W. A.; Drier, Y.; Johnstone, S. E.; et al. Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. *Nature* **2019**, *575*, 229–233.
- [19] Nguyen, L.; Van Hoeck, A.; Cuppen, E. Machine learning-based tissue of origin classification for cancer of unknown primary

- diagnostics using genome-wide mutation features. *Nat. Commun.* **2022**, *13*, 4013.
- [20] Liu, Y. T.; Wang, Y. L.; Wang, S.; et al. Turning cold tumors into hot tumors to ignite immunotherapy. *Mol. Cancer* **2025**, *24*, 254.
- [21] Song, J. W.; Yang, P.; Chen, C. T.; et al. Targeting epigenetic regulators as a promising avenue to overcome cancer therapy resistance. *Signal Transduct. Target. Ther.* **2025**, *10*, 219.
- [22] Zhang, Y.; Naderi Yeganeh, P.; Zhang, H. W.; et al. Tumor editing suppresses innate and adaptive antitumor immunity and is reversed by inhibiting DNA methylation. *Nat. Immunol.* **2024**, *25*, 1858–1870.
- [23] Kagan, A. B.; Garrison, D. A.; Anders, N. M.; et al. DNA methyltransferase inhibitor exposure–response: Challenges and opportunities. *Clin. Transl. Sci.* **2023**, *16*, 1309–1322.
- [24] Marci, H. E. Epigenetic regulators in cancer therapy and progression. *Npj Precis. Oncol.* **2025**, *9*, 206.
- [25] Hsu, P. D.; Lander, E. S.; Zhang, F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* **2014**, *157*, 1262–1278.
- [26] McDonald, J. I.; Celik, H.; Rois, L. E.; et al. Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. *Biol. Open* **2016**, *5*, 866–874.
- [27] Pickar-Oliver, A.; Gersbach, C. A. The next generation of CRISPR-Cas technologies and applications. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 490–507.
- [28] McCutcheon, S. R.; Rohm, D.; Iglesias, N.; et al. Epigenome editing technologies for discovery and medicine. *Nat. Biotechnol.* **2024**, *42*, 1199–1217.
- [29] Yuan, L. W.; Xiong, Y. K.; Zhang, Y. M.; et al. Epigenome editing based treatment: Progresses and challenges. *Mol. Ther.* **2026**, *34*, 46–67.
- [30] Xu, C.; Zeng, C.; Wang, M. G.; et al. mRNA-engineered CRISPR-Cas epigenetic editors enable durable and efficient gene silencing *in vivo*. *Innovation* **2026**, *7*, 101151.
- [31] Vojta, A.; Dobrinic, P.; Tadić, V.; et al. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Res.* **2016**, *44*, 5615–5628.
- [32] Amabile, A.; Migliara, A.; Capasso, P.; et al. Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. *Cell* **2016**, *167*, 219–232.e14.
- [33] O’Green, H.; Tomkova, M.; Combs, J. A.; et al. Determinants of heritable gene silencing for KRAB-dCas9 + DNMT3 and Ezh2-dCas9 + DNMT3 hit-and-run epigenome editing. *Nucleic Acids Res.* **2022**, *50*, 3239–3253.
- [34] Lin, K. T.; Zou, C.; Hubbard, A.; et al. Multiplexed epigenetic memory editing using CRISPRoff sensitizes glioblastoma to chemotherapy. *Neuro Oncol.* **2025**, *27*, 1443–1457.
- [35] Han, X. Y.; Abdallah, M. O. E.; Breuer, P.; et al. Downregulation of MGMT expression by targeted editing of DNA methylation enhances temozolomide sensitivity in glioblastoma. *Neoplasia* **2023**, *44*, 100929.
- [36] Kouroukli, A. G.; Rajaram, N.; Bashtrykov, P.; et al. Targeting oncogenic TERT promoter variants by allele-specific epigenome editing. *Clin. Epigenet.* **2023**, *15*, 183.
- [37] He, W. H.; Zhu, H. J.; Zhang, S. F.; et al. Epigenetic editing of BRCA1 promoter increases cisplatin and olaparib sensitivity of ovarian cancer cells. *Epigenetics* **2024**, *19*, 2357518.
- [38] Xu, C.; Jiang, S. Y.; Ma, X. Y.; et al. CRISPR-based DNA methylation editing of NNT rescues the cisplatin resistance of lung cancer cells by reducing autophagy. *Arch. Toxicol.* **2023**, *97*, 441–456.
- [39] Zahraei, M.; Azimi, Y.; Karimipour, M.; et al. CRISPR/dCas9-TET1-mediated epigenetic editing reactivates miR-200c in breast cancer cells. *Sci. Rep.* **2025**, *15*, 31739.
- [40] Zhang, W. F.; Zhao, J.; Fan, X. T.; et al. Targeted demethylation of the EphA7 promoter inhibits tumorigenesis *via* the SP1/DNMT1 and PI3K/AKT axes and improves the response to multiple therapies in cervical cancer. *Cell Death Dis.* **2025**, *16*, 324.
- [41] Choudhury, S. R.; Cui, Y.; Lubecka, K.; et al. CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at *BRCA1* promoter. *Oncotarget* **2016**, *7*, 46545–46556.
- [42] Goudy, L.; Ha, A.; Borah, A. A.; et al. Integrated epigenetic and genetic programming of primary human T cells. *Nat. Biotechnol.* **2025**, 1–12.
- [43] Tejedor, J. R.; Peñarroya, A.; Gancedo-Verdejo, J.; et al. CRISPR/dCas9-mediated DNA demethylation screen identifies functional epigenetic determinants of colorectal cancer. *Clin. Epigenet.* **2023**, *15*, 133.
- [44] Sgro, A.; Blancafort, P. Epigenome engineering: New technologies for precision medicine. *Nucleic Acids Res* **2020**, *48*, 12453–12482.
- [45] Lei, T.; Wang, Y. Z.; Zhang, Y. C.; et al. Leveraging CRISPR gene editing technology to optimize the efficacy, safety and accessibility of CAR T-cell therapy. *Leukemia* **2024**, *38*, 2517–2543.
- [46] Stylianopoulos, T.; Munn, L. L.; Jain, R. K. Reengineering the physical microenvironment of tumors to improve drug delivery and efficacy: From mathematical modeling to bench to bedside. *Trends Cancer* **2018**, *4*, 292–319.
- [47] Boucher, P.; Cui, X. X.; Curiel, D. T. Adenoviral vectors for *in vivo* delivery of CRISPR-Cas gene editors. *J. Control. Release* **2020**, *327*, 788–800.
- [48] Saito, M.; Xu, P. Y.; Faure, G.; et al. Fanzor is a eukaryotic programmable RNA-guided endonuclease. *Nature* **2023**, *620*, 660–668.
- [49] Jiang, K. Y.; Lim, J.; Sgrizzi, S.; et al. Programmable RNA-guided DNA endonucleases are widespread in eukaryotes and their viruses. *Sci. Adv.* **2023**, *9*, eadk0171.
- [50] Wei, Y. H.; Gao, P. F.; Pan, D.; et al. Engineering eukaryotic transposon-encoded Fanzor2 system for genome editing in mammals. *Nat. Chem. Biol.* **2026**, *22*, 48–57.
- [51] Song, Z. G.; Guo, J. F.; Fan, Z. Q.; et al. Noncanonical target-strand cytosine base editing *via* engineered UnlCas12f1 platform. *Nat. Commun.* **2025**, *16*, 9499.
- [52] Xu, P. Y.; Saito, M.; Faure, G.; et al. Structural insights into the diversity and DNA cleavage mechanism of Fanzor. *Cell* **2024**, *187*, 5238–5252.e20.
- [53] Qiu, M.; Glass, Z.; Chen, J. J.; et al. Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific *in vivo* genome editing of *Angptl3*. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, e2020401118.
- [54] Cheng, Q.; Wei, T.; Farbiak, L.; et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat. Nanotechnol.* **2020**, *15*, 313–320.
- [55] Abaza, T.; Mohamed, E. E.; Zaky, M. Y. Lipid nanoparticles: A promising tool for nucleic acid delivery in cancer immunotherapy. *Med. Oncol.* **2025**, *42*, 409.
- [56] Duan, Z. Q.; Liang, Y. F.; Sun, J. L.; et al. An engineered Cas12i nuclease that is an efficient genome editing tool in animals and plants. *Innovation* **2024**, *5*, 100564.
- [57] Xu, X. S.; Chemparathy, A.; Zeng, L. P.; et al. Engineered miniature CRISPR-Cas system for mammalian genome regulation and editing. *Mol. Cell* **2021**, *81*, 4333–4345.e4.
- [58] Butterfield, G. L.; Gersbach, C. A. Resurrecting a miniature Cas9 ancestor for genome and epigenome editing. *Nat. Biotechnol.* **2025**, 1–2.
- [59] Neumann, E. N.; Bertozzi, T. M.; Wu, E.; et al. Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor. *Science* **2024**, *384*, ado7082.
- [60] Lu, M.; Xing, H. N.; Zhao, X. Y.; et al. Engineered extracellular vesicles as a next-generation vaccine platform. *Matter* **2024**, *7*, 4180–4205.
- [61] Xu, D.; Besselink, S.; Ramadoss, G. N.; et al. Programmable epigenome editing by transient delivery of CRISPR epigenome editor ribonucleoproteins. *Nat. Commun.* **2025**, *16*, 7948.

- [62] Tycko, J.; Wainberg, M.; Marinov, G. K.; et al. Mitigation of off-target toxicity in CRISPR-Cas9 screens for essential non-coding elements. *Nat. Commun.* **2019**, *10*, 4063.
- [63] Kleinstiver, B. P.; Pattanayak, V.; Prew, M. S.; et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature* **2016**, *529*, 490–495.
- [64] Vakulskas, C. A.; Dever, D. P.; Rettig, G. R.; et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. *Nat. Med.* **2018**, *24*, 1216–1224.
- [65] Galonska, C.; Charlton, J.; Mattei, A. L.; et al. Genome-wide tracking of dCas9-methyltransferase footprints. *Nat. Commun.* **2018**, *9*, 597.
- [66] Pflueger, C.; Tan, D.; Swain, T.; et al. A modular dCas9-SunTag DNMT3A epigenome editing system overcomes pervasive off-target activity of direct fusion dCas9-DNMT3A constructs. *Genome Res.* **2018**, *28*, 1193–1206.
- [67] Stepper, P.; Kungulovski, G.; Jurkowska, R. Z.; et al. Efficient targeted DNA methylation with chimeric dCas9-Dnmt3a-Dnmt3L methyltransferase. *Nucleic Acids Res.* **2017**, *45*, 1703–1713.
- [68] Vizoso, M.; van Rheenen, J. Diverse transcriptional regulation and functional effects revealed by CRISPR/Cas9-directed epigenetic editing. *Oncotarget* **2021**, *12*, 1651–1662.
- [69] Sayar, E.; Patel, R. A.; Coleman, I. M.; et al. Reversible epigenetic alterations mediate PSMA expression heterogeneity in advanced metastatic prostate cancer. *JCI Insight* **2023**, *8*, e162907.
- [70] Klann, T. S.; Black, J. B.; Chellappan, M.; et al. CRISPR-Cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome. *Nat. Biotechnol.* **2017**, *35*, 561–568.
- [71] Pierce, S. E.; Granja, J. M.; Greenleaf, W. J. High-throughput single-cell chromatin accessibility CRISPR screens enable unbiased identification of regulatory networks in cancer. *Nat. Commun.* **2021**, *12*, 2969.
- [72] Ruffolo, J. A.; Nayfach, S.; Gallagher, J.; et al. Design of highly functional genome editors by modelling CRISPR-Cas sequences. *Nature* **2025**, *645*, 518–525.
- [73] Nguyen, E.; Poli, M.; Durrant, M. G.; et al. Sequence modeling and design from molecular to genome scale with Evo. *Science* **2024**, *386*, eado9336.
- [74] Brixi, G.; Durrant, M. G.; Ku, J.; et al. Genome modelling and design across all domains of life with Evo 2. *Nature* **2026**, *1*–13.
- [75] Tran, V. Q.; Nemeth, M.; Bartie, L. J.; et al. Rapid directed evolution guided by protein language models and epistatic interactions. *Science* **2026**, eaeal820.
- [76] Dauparas, J.; Anishchenko, I.; Bennett, N.; et al. Robust deep learning-based protein sequence design using ProteinMPNN. *Science* **2022**, *378*, 49–56.
- [77] Watson, J. L.; Juergens, D.; Bennett, N. R.; et al. *De novo* design of protein structure and function with RFdiffusion. *Nature* **2023**, *620*, 1089–1100.
- [78] Xu, Y.; Cui, H. T.; Pang, K.; et al. LUMI-lab: A foundation model-driven autonomous platform enabling discovery of ionizable lipid designs for mRNA delivery. *Cell* **2026**, *189*, 1620–1635.e25.
- [79] Cappelluti, M. A.; Mollica Poeta, V.; Valsoni, S.; et al. Durable and efficient gene silencing *in vivo* by hit-and-run epigenome editing. *Nature* **2024**, *627*, 416–423.
- [80] Stanojević, D.; Li, Z.; Bakić, S.; et al. Rockfish: A transformer-based model for accurate 5-methylcytosine prediction from nanopore sequencing. *Nat. Commun.* **2024**, *15*, 5580.
- [81] Bai, X.; Yao, H. C.; Wu, B.; et al. DeepBAM: A high-accuracy single-molecule CpG methylation detection tool for Oxford nanopore sequencing. *Brief. Bioinform.* **2024**, *25*, bbae413.
- [82] Nichols, R. V.; Rylaarsdam, L. E.; O'Connell, B. L.; et al. Atlas-scale single-cell DNA methylation profiling with sciMETv3. *Cell Genom.* **2025**, *5*, 100726.
- [83] SeekGene BioSciences. SeekOne™ DD Single-Cell Multiome Methylation + RNA Kit (scMethyl + RNA-seq): SeekGene BioSciences; 2026 [Available from: <https://www.seekgene.com/Product/scMethyl-RNA-seq.html>].
- [84] Javed, N.; Weingarten, T.; Sehanobish, A.; et al. A multi-modal transformer for cell type-agnostic regulatory predictions. *Cell Genom.* **2025**, *5*, 100762.
- [85] Yan, F. Y.; Telonis, A. G.; Yang, Q.; et al. Genome-wide methylome modeling *via* generative AI incorporating long- and short-range interactions. *Sci. Adv.* **2025**, *11*, eadt4152.
- [86] Yan, Y. Y.; Chai, X. Y.; Liu, J. J.; et al. DeepMethyGene: A deep-learning model to predict gene expression using DNA methylations. *BMC Bioinform.* **2025**, *26*, 99.
- [87] Avsec, Ž.; Latysheva, N.; Cheng, J.; et al. Advancing regulatory variant effect prediction with AlphaGenome. *Nature* **2026**, *649*, 1206–1218.
- [88] Avsec, Ž.; Agarwal, V.; Visentin, D.; et al. Effective gene expression prediction from sequence by integrating long-range interactions. *Nat. Meth.* **2021**, *18*, 1196–1203.
- [89] Kamimoto, K.; Stringa, B.; Hoffmann, C. M.; et al. Dissecting cell identity *via* network inference and in silico gene perturbation. *Nature* **2023**, *614*, 742–751.
- [90] Theodoris, C. V.; Xiao, L.; Chopra, A.; et al. Transfer learning enables predictions in network biology. *Nature* **2023**, *618*, 616–624.
- [91] Cui, H. T.; Wang, C.; Maan, H.; et al. scGPT: Toward building a foundation model for single-cell multi-omics using generative AI. *Nat. Meth.* **2024**, *21*, 1470–1480.
- [92] Bravo González-Blas, C.; De Winter, S.; Hulselmanns, G.; et al. SCENIC+ single-cell multiomic inference of enhancers and gene regulatory networks. *Nat. Methods* **2023**, *20*, 1355–1367.
- [93] Jones, P. A.; Baylin, S. B. The epigenomics of cancer. *Cell* **2007**, *128*, 683–692.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits reusers to distribute, remix, adapt, and build upon the material in any medium or format, so long as attribution is given to the original author(s) and the source, provide a link to the license, and indicate if changes were made. See <https://creativecommons.org/licenses/by/4.0/>

© The author(s) 2026. Published by Tsinghua University Press.