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Epigenetic therapies in cancer treatment: Opportunities and challenges

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Abstract

Epigenetic therapies offer a revolutionary approach to cancer treatment by targeting reversible modifications such as DNA methylation, histone modifications, and non-coding RNAs that regulate gene expression. FDA-approved drugs like DNA methyltransferase inhibitors and histone deacetylase inhibitors have demonstrated clinical efficacy, particularly in hematologic malignancies, while emerging agents like BET and EZH2 inhibitors show promise in addressing therapeutic gaps in solid tumors. This review delves into these advancements, highlighting the integration of epigenetic drugs with immunotherapy, chemotherapy, and targeted therapies to enhance therapeutic precision and overcome resistance. Moreover, it emphasizes opportunities such as biomarker-driven personalization and liquid biopsy technologies for real-time monitoring, alongside innovations like single-cell epigenomics and CRISPR-based tools that facilitate tumor heterogeneity studies and precise therapeutic interventions. Despite these developments, challenges such as off-target effects, delivery inefficiencies, and regulatory hurdles persist, necessitating further innovation. By integrating epigenetic therapies with RNA-based drugs, nanomedicine, personalized vaccines, 3D epigenomics, and artificial intelligence, this review underscores the transformative potential of these therapies in redefining oncology, offering personalized, durable, and precise solutions to the complexities of cancer.

Keywords: Epigenetic therapies; Cancer treatment; DNA methylation; Histone modifications; Non-coding RNAs; Tumor microenvironment

1. Introduction

1.1. Overview of Epigenetics in Cancer

The concept of epigenetics; though widely discussed today; has a rich and evolving history that reflects the progression of scientific understanding. The term "epigenetics" was first introduced in 1942 by Conrad Waddington; who sought to describe the complex interactions between genes and their surrounding environment that influence development and cellular differentiation. Waddington's famous "epigenetic landscape" metaphor illustrated the paths a cell could take during differentiation; with external and internal cues shaping its trajectory [1;2]. His work laid the foundation for recognizing that gene expression could be modified without altering the underlying DNA sequence [2].

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By the 1970s and 1980s; significant breakthroughs in molecular biology further defined the mechanisms of epigenetic regulation. The pioneering work of Adrian Bird and Robin Holliday; among others; established the role of DNA methylation in gene silencing [3;4]. Simultaneously; researchers like David Allis and colleagues [5] uncovered the significance of histone modifications; including acetylation and methylation; in chromatin structure and gene expression. These studies revealed that specific chemical tags could dynamically regulate the accessibility of DNA to transcriptional machinery; providing a mechanism for cellular memory and differentiation [5].

The discovery of non-coding RNAs (ncRNAs) in the late 20th century added another layer to the understanding of epigenetic regulation. Initially dismissed as "junk;" these RNAs were later shown to play critical roles in modulating gene expression at both transcriptional and post-transcriptional levels. Small ncRNAs like microRNAs (miRNAs) and long ncRNAs (lncRNAs) emerged as key players in controlling diverse cellular processes; including those implicated in cancer progression [6].

The modern era of epigenetics research has been marked by advancements in high-throughput sequencing technologies; enabling the detailed mapping of epigenomic landscapes across different cell types and disease states. These developments have not only deepened the understanding of epigenetic mechanisms but also highlighted their relevance in various diseases; particularly cancer; where aberrant epigenetic modifications contribute to oncogenesis; metastasis; and treatment resistance [7;8].

1.2. Transition to Contemporary Perspectives

Building on this historical foundation; the contemporary study of epigenetics emphasizes its clinical implications; especially in oncology. The dynamic and reversible nature of epigenetic modifications offers unique opportunities for therapeutic intervention [9;10]. Understanding how dysregulated DNA methylation; histone modifications; and non-coding RNAs contribute to tumorigenesis forms the basis for developing targeted epigenetic therapies. This review explores these mechanisms; current therapeutic advancements; and the challenges associated with their clinical application; ultimately aiming to illuminate the transformative potential of epigenetic therapies in cancer treatment [10].

Epigenetics; the study of heritable changes in gene expression that occur without alterations to the DNA sequence; plays a central role in cellular identity and function. This regulatory system is orchestrated by three major mechanisms: DNA methylation; histone modifications; and non-coding RNAs (ncRNAs) [11;12]. DNA methylation; mediated by enzymes such as DNA methyltransferases; predominantly acts to repress gene expression by adding methyl groups to cytosine bases in CpG dinucleotides; often silencing tumor suppressor genes in cancer. Conversely; histone modifications; including acetylation and methylation; regulate chromatin structure and accessibility; influencing transcriptional activity. Aberrant patterns of these modifications have been implicated in activating oncogenes or silencing key regulatory genes; contributing to tumorigenesis [12]. Non-coding RNAs; including microRNAs and long non-coding RNAs; further modulate gene expression by interacting with chromatin or regulating transcription and translation. Together; these mechanisms create a complex regulatory landscape that maintains cellular homeostasis but can be disrupted in cancer; driving disease progression and metastasis [13;14].

Epigenetic dysregulation in cancer not only facilitates malignant transformation but also promotes adaptability and resistance to therapies; underscoring its significance in tumor heterogeneity and plasticity. Unlike genetic mutations; which are permanent; epigenetic changes are reversible; making them particularly attractive therapeutic targets. Advances in understanding these mechanisms have paved the way for epigenetic therapies; such as inhibitors targeting DNA methylation and histone deacetylation; which aim to restore normal gene expression and re-sensitize tumors to conventional treatments. This paradigm shift emphasizes the potential of epigenetic therapies to address critical challenges in oncology; such as treatment resistance and metastasis [14].

1.3. Scope and Objectives of the Review

This review explores the intricate mechanisms underlying epigenetic therapies; detailing how disruptions in DNA methylation; histone modifications; and non-coding RNA pathways contribute to cancer development. The discussion extends to existing epigenetic drugs; their clinical applications; and the challenges of developing more selective and efficacious therapeutics. By highlighting emerging technologies; such as CRISPR-based epigenome editing and single-cell epigenomics; this review aims to present a comprehensive outlook on the future directions of this rapidly evolving field.

The objectives are twofold: first; to synthesize current knowledge on the therapeutic potential of targeting epigenetic mechanisms; and second; to critically examine the barriers to their successful clinical translation. By doing so; this

article seeks to provide insights into the opportunities and challenges that define the role of epigenetic therapies in modern oncology.

2. Epigenetic Mechanisms and Their Role in Cancer

The regulation of gene expression through epigenetic mechanisms plays a critical role in cellular development and cancer progression. Unlike genetic mutations, which involve changes to the DNA sequence itself, epigenetic modifications alter how genes are expressed without changing the underlying genetic code. This makes epigenetics a potentially reversible mechanism, offering a unique avenue for therapeutic intervention in cancer treatment [15]. This section outlines the mechanisms by which environmental factors influence epigenetic processes, thereby modulating gene expression (Figure 1) (Table 1). The primary epigenetic mechanisms in cancer involve DNA methylation, histone modifications, and non-coding RNAs. Here, we focus on the first of these—DNA methylation—and its significant role in tumorigenesis, as well as the therapeutic potential of targeting this process [15,16].

Table 1 Epigenetic Mechanisms in Cancer: Roles, Examples, and Impacts

Mechanism	Role	Examples	Impact on Gene Expression
DNA Methylation	Addition of methyl groups to cytosine bases in CpG islands, typically repressing transcription.	Tumor suppressor gene hypermethylation (e.g., p16INK4A, MLH1)	Silences genes critical for DNA repair and cell cycle control, facilitating tumorigenesis. Hypomethylation activates oncogenes like c-MYC.
Histone Modifications	Post-translational changes to histone proteins influencing chromatin structure and gene accessibility.	Acetylation by HATs activates transcription; Deacetylation by HDACs represses it. Methylation by EZH2 at H3K27 leads to repression.	Dysregulation can activate oncogenes or repress tumor suppressors, contributing to metastasis, therapy resistance, and uncontrolled proliferation.
Non-Coding RNAs	Regulate transcription and translation through RNA-RNA or RNA-DNA interactions.	miRNAs (e.g., miR-21, miR-34a); lncRNAs (e.g., HOTAIR, MALAT1)	Oncogenic miRNAs suppress tumor suppressor genes, while tumor-suppressive miRNAs are downregulated in cancers. lncRNAs modulate chromatin to support cancer growth.

2.1. DNA Methylation

DNA methylation is a key epigenetic modification that involves the addition of a methyl group to the 5' position of cytosine residues, typically within CpG dinucleotides. This process plays a fundamental role in regulating gene expression, with methylation often leading to gene silencing. In the context of cancer, both hypermethylation and hypomethylation are commonly observed and contribute to tumorigenesis in distinct ways.

2.1.1. Hyper/hypomethylation in Oncogenes and Tumor Suppressor Genes

Hypermethylation of promoter regions of tumor suppressor genes (TSGs) is a well-established mechanism that silences these critical genes in cancer cells. For example, the hypermethylation of genes like p16INK4A and MLH1 leads to their silencing, contributing to the loss of cell cycle control and DNA repair mechanisms, respectively, thereby promoting uncontrolled cellular proliferation and accumulation of mutations [17,18].

Conversely, hypomethylation is often observed in the promoter regions of oncogenes, such as c-MYC and RAS, which are typically upregulated in cancer cells. Hypomethylation can lead to the activation of these normally silenced genes, enhancing cell survival, proliferation, and migration—hallmarks of cancer. This aberrant activation is particularly common in cancers such as colorectal, breast, and pancreatic cancer, where widespread hypomethylation across the genome contributes to genomic instability and oncogene activation [18].

These two opposing methylation patterns—hypermethylation of TSGs and hypomethylation of oncogenes—are crucial to the development of cancer. They not only disrupt normal gene regulation but also provide a snapshot of the altered epigenetic landscape of tumors, which is useful for early diagnosis and prognostic prediction [15-18].

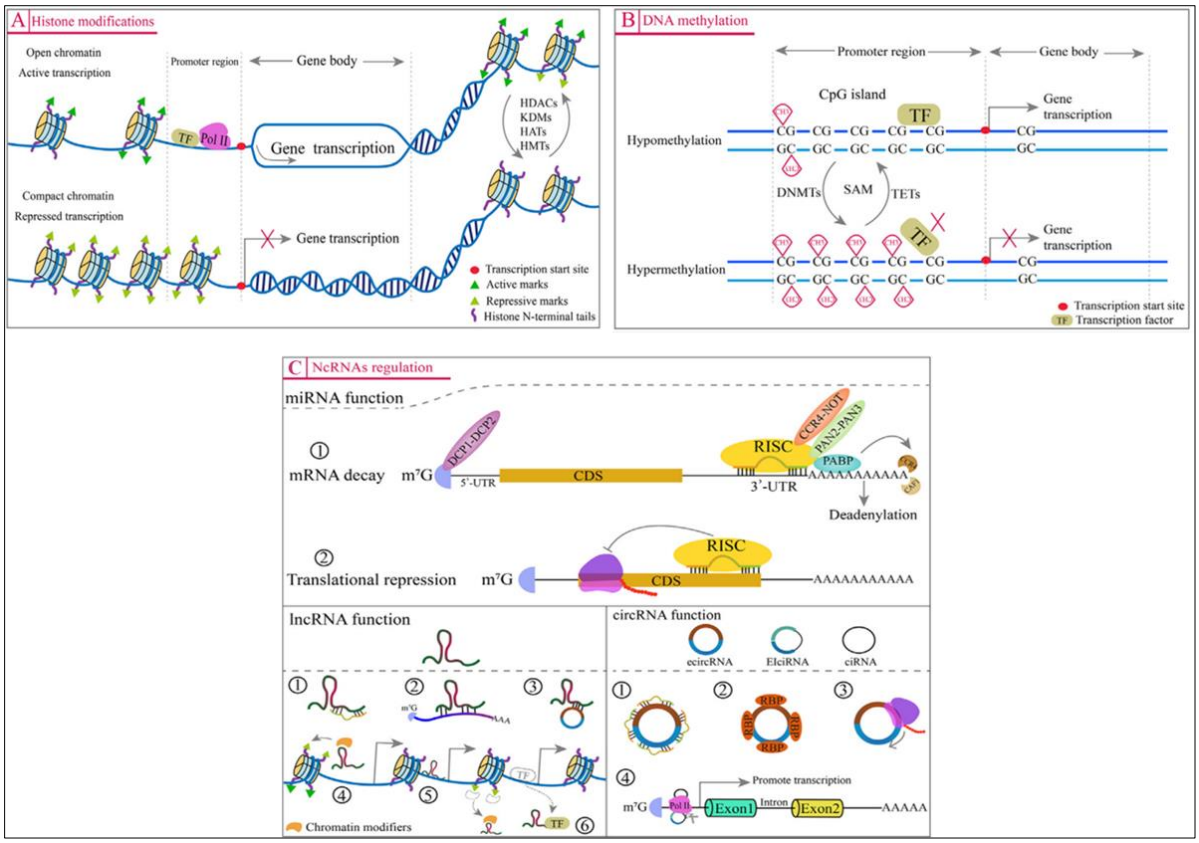

Figure 1 A schematic diagram illustrating the three major epigenetic mechanisms (DNA methylation, histone modifications, and non-coding RNAs) with their roles in regulating gene expression. Reproduced from Ref. [18] with permission, copyright, Elsevier 2021

2.1.2. Methylation as a Therapeutic Target

Given the pivotal role of DNA methylation in cancer, it has become an attractive target for therapeutic intervention. The most well-known approach to targeting DNA methylation in cancer therapy involves the use of DNA methyltransferase inhibitors (DNMTis), such as 5-azacytidine (5-Aza) and decitabine. These agents work by inhibiting the activity of DNMTs, the enzymes responsible for adding methyl groups to DNA. By blocking DNMT activity, these drugs can reactivate silenced tumor suppressor genes, thereby restoring normal cell function and inducing cancer cell death [16,19].

In addition to their potential in reactivating silenced genes, DNMT inhibitors can also restore the immune system's ability to recognize and attack tumor cells. For example, in hematologic cancers such as leukemia, DNMT inhibitors have been shown to enhance immune cell responses by demethylating genes involved in immune surveillance. However, the use of DNMT inhibitors in solid tumors has faced challenges, including poor tumor penetration and the complex nature of the tumor microenvironment. Nonetheless, the combination of DNMT inhibitors with other therapeutic modalities, such as immunotherapies or targeted therapies, is being explored to enhance their effectiveness in solid tumors [20].

Moreover, research has highlighted the dual role of DNMTs in cancer. According to Li et al. [21] while DNMT1 is typically associated with promoting cancer cell survival, its deficiency in certain contexts, such as in prostate cancer, has been linked to the promotion of cancer stem cell phenotypes and enhanced epithelial-mesenchymal transition (EMT), further complicating the therapeutic landscape. This dual nature underscores the need for precise targeting and the careful design of therapeutic strategies that take into account the specific methylation profiles of different cancer types [21].

Overall, the therapeutic targeting of DNA methylation holds great promise but also presents significant challenges. The development of more selective inhibitors, as well as combination strategies with other therapies, will likely be key to overcoming these hurdles and improving the clinical outcomes of cancer patients.

2.2. Histone Modifications

The nucleosome core particle, the fundamental unit of chromatin, consists of 147 base pairs of DNA wrapped around an octamer of the four core histone proteins in a 1.7 left-handed superhelical turn. The positively charged histone proteins bind efficiently to the negatively charged phosphate backbone of DNA, stabilizing the structure. The four core histones, H2A, H2B, H3, and H4, are organized within the nucleosome as an H2A-H2B dimer and an H3-H4 tetramer, stabilized by the linker histone H1, which connects adjacent nucleosomes. While the amino acid sequences of histones show significant variation across species, they share a conserved structural domain known as the histone fold. This domain consists of a long central helix flanked by two helix-strand-helix motifs at its ends. Additionally, the N-terminal tails of histones are highly flexible and enriched with lysine and arginine residues, which are primary sites for extensive post-translational modifications by various cellular mechanisms (Figure 2). Histone modifications are critical post-translational changes to histone proteins that regulate chromatin structure and gene expression without altering the underlying DNA sequence. These modifications, which include acetylation, methylation, phosphorylation, and ubiquitination, play a pivotal role in determining whether a gene is transcriptionally active or repressed. Dysregulation of these processes has been extensively linked to cancer progression, influencing oncogenesis, metastasis, and therapy resistance [22]. Understanding these modifications and their regulatory enzymes offers significant opportunities for therapeutic intervention in cancer treatment.

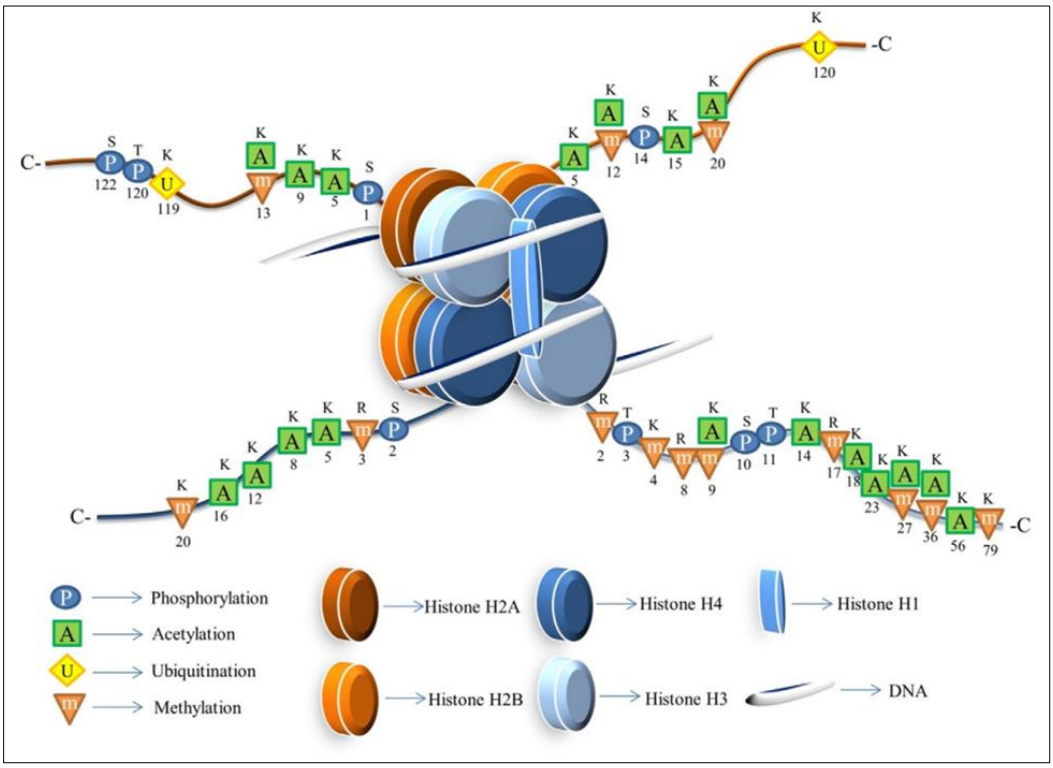

Figure 2 Molecular and Structural features of histones which are foundational to understanding their modifications. Reproduced from Ref. [25] with permission, copyright, Elsevier 2017

2.2.1. Acetylation, Methylation, Phosphorylation, and Ubiquitination

Histone acetylation involves the addition of acetyl groups to lysine residues on histone tails, catalyzed by histone acetyltransferases (HATs). This modification neutralizes the positive charge on histones, leading to a relaxed chromatin structure that promotes transcription. Conversely, histone deacetylases (HDACs) remove acetyl groups, resulting in chromatin compaction and transcriptional repression [23]. Dysregulated acetylation is a hallmark of several cancers, such as breast and colorectal cancers, where HDAC overexpression silences tumor suppressor genes.

Histone methylation, catalyzed by histone methyltransferases (HMTs), adds methyl groups to specific lysine or arginine residues. This modification can have diverse effects depending on the site and degree of methylation. For instance, from the findings of Gupta et al. [24] trimethylation of histone H3 lysine 4 (H3K4me3) is associated with gene activation, while trimethylation of lysine 9 (H3K9me3) is linked to gene repression. Aberrant methylation patterns are implicated

in the activation of oncogenes and the silencing of tumor suppressor genes, driving cancer progression in various malignancies, including leukemia and lung cancer [23-26].

Phosphorylation of histones, often mediated by kinases in response to DNA damage or cell stress, regulates chromatin dynamics and facilitates repair mechanisms. Dysregulated histone phosphorylation can impair DNA repair pathways, contributing to genomic instability in cancers such as glioblastoma.

Ubiquitination, the attachment of ubiquitin molecules to histones, influences chromatin remodeling and gene expression. Monoubiquitination of histone H2B is associated with transcriptional activation, while polyubiquitination often signals histone degradation. Alterations in histone ubiquitination pathways are observed in prostate and breast cancers, where they disrupt normal chromatin organization and transcriptional fidelity [24,26].

2.2.2. Role of Histone-Modifying Enzymes in Cancer Progression

Histone-modifying enzymes serve as crucial regulators of the epigenetic procedures, influencing gene expression through the addition, removal, or recognition of post-translational histone marks. In cancer, the dysregulation of these enzymes disrupts normal transcriptional control, contributing to the development and progression of malignancies. Common enzymes are discussed below.

Histone Deacetylases (HDACs)

Histone deacetylases remove acetyl groups from lysine residues on histones, leading to chromatin compaction and transcriptional repression. Overexpression of HDACs is commonly observed in cancers such as breast, colorectal, and lung cancers, where it silences tumor suppressor genes like p21 and RB1 [23,27]. According to Karagiannis et al. [28], HDAC inhibitors (HDACis), such as vorinostat (SAHA) and romidepsin, effectively restore gene expression by blocking HDAC activity. These agents have shown particular promise in treating hematologic malignancies like cutaneous T-cell lymphoma [28].

Histone Methyltransferases (HMTs)

HMTs catalyze the addition of methyl groups to lysine or arginine residues on histones, with their activity being highly site-specific. For instance, EZH2, a component of the Polycomb repressive complex 2 (PRC2), adds methyl groups to histone H3 at lysine 27 (H3K27me3), leading to gene silencing. In prostate and breast cancers, EZH2 is often overexpressed, contributing to tumor growth and metastasis. Inhibitors targeting EZH2, such as tazemetostat, are under clinical investigation and have demonstrated efficacy in reducing tumor burden in cancers with mutations in SWI/SNF chromatin-remodeling complexes [29,30].

Histone Demethylases (HDMs)

HDMs remove methyl groups from histones, reversing the effects of HMTs. One key enzyme, lysine-specific demethylase 1 (LSD1), regulates transcription by demethylating H3K4me1/me2. Overactivation of LSD1 in cancers like acute myeloid leukemia (AML) promotes the silencing of differentiation-related genes. Targeting LSD1 with inhibitors such as iadademstat is emerging as a novel strategy to restore normal differentiation and inhibit cancer progression [31,32].

Interplay with Other Epigenetic Mechanisms

These enzymes often interact with other epigenetic regulators, including DNA methyltransferases and non-coding RNAs, forming complex networks that sustain the oncogenic state. For instance, according to Cai et al. [33], HDACs collaborate with DNMTs to establish repressive chromatin environments, silencing tumor suppressor genes synergistically. Therapeutic strategies targeting these interactions are currently being explored to enhance treatment efficacy [33]

By elucidating the roles of histone-modifying enzymes such as HDACs, HMTs, and HDMs, researchers aim to develop targeted therapies that disrupt the epigenetic drivers of cancer. These efforts have already begun to yield promising results, with epigenetic drugs entering clinical use and offering hope for more effective cancer treatments [34].

2.3. Non-Coding RNAs

Non-coding RNAs (ncRNAs), encompassing microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play a pivotal role in the epigenetic regulation of gene expression. Unlike messenger RNAs, ncRNAs do not encode proteins but function as crucial regulators of transcriptional and post-transcriptional processes. Their dysregulation is frequently observed in cancers, where they contribute to tumor progression, metastasis, and therapy resistance [14,35]

2.3.1. The Role of miRNAs and lncRNAs in Epigenetic Regulation

MicroRNAs, typically 20-25 nucleotides long, act as potent gene silencers by binding to the 3' untranslated regions (UTRs) of target mRNAs, leading to their degradation or translational inhibition. Dysregulated miRNAs can act as oncogenes or tumor suppressors depending on their target genes [36]. Research findings by Abulsoud et al. [37] indicate that miR-34a, a well-known tumor suppressor, inhibits pathways involved in cell proliferation, while oncogenic miRNAs like miR-21 enhance tumor growth by suppressing tumor suppressor genes such as PTEN. Aberrant miRNA expression is strongly linked to cancer initiation and progression across various types, including breast, colorectal, and lung cancers [36,37].

Long non-coding RNAs, typically longer than 200 nucleotides, function through diverse mechanisms, including chromatin remodeling, acting as decoys for miRNAs, or scaffolding protein complexes. LncRNAs such as HOTAIR and MALAT1 have been implicated in modifying histone marks and chromatin states, thereby promoting oncogenic transcriptional programs. For instance, from the notable research work carried out by Wu et al. [38], it is observed that HOTAIR interacts with the Polycomb Repressive Complex 2 (PRC2) to mediate gene silencing in tumor suppressor pathways. Conversely, tumor-suppressive lncRNAs like GAS5 sequester glucocorticoid receptors to inhibit cancer cell survival signaling [38-40].

2.3.2. Potential for Targeting Non-Coding RNAs in Therapy

The involvement of ncRNAs in critical cancer pathways makes them promising therapeutic targets. MiRNA-based therapies include the use of miRNA mimics to restore tumor-suppressive activity or anti-miRNA oligonucleotides (antagomirs) to inhibit oncogenic miRNAs [41]. For example, miR-34a mimics have shown potential in preclinical trials for inducing cancer cell apoptosis.

Similarly, targeting lncRNAs is gaining traction. Antisense oligonucleotides (ASOs) can selectively inhibit oncogenic lncRNAs, while small molecules are being explored to disrupt lncRNA-protein interactions. A notable example is targeting HOTAIR to reverse its oncogenic effects. Emerging delivery systems, including lipid nanoparticles and exosomes, enhance the stability and specificity of these RNA-based therapies in vivo. Research findings from Eisa et al. [42] explore the anti-carcinogenic effects of diacerein (DIA) in colorectal cancer (CRC). The study demonstrated that DIA disrupts key molecular pathways involved in CRC progression, including the IL-6/STAT3/HOTAIR axis, Wnt/β-Catenin, and TLR4/NF-κB/TNF-α pathways. DIA treatment induced apoptosis, inhibited tumor angiogenesis, and downregulated critical signaling proteins, suggesting its potential as an effective therapeutic strategy for CRC management. This work underscores DIA's role in modulating inflammation-driven pathways in cancer [42]. Likewise, research findings from Naveed et al. [43] demonstrate the functional diversity of NEAT1 lncRNA isoforms in neuroblastoma using polyA-modulating antisense oligonucleotides (ASOs). These ASOs shift the NEAT1 isoform balance, reducing NEAT1_1 and enhancing NEAT1_2, which promotes paraspeckle formation. This results in reduced tumor cell proliferation and increased differentiation, highlighting the therapeutic potential of isoform-specific lncRNA modulation. The study underscores ASOs as a promising tool to target lncRNA dynamics in cancer therapy [43]. Additionally, the crosstalk between miRNAs and lncRNAs represents an intricate regulatory network that can be exploited for combinatorial treatments. For example, lncRNA MALAT1 sponges tumor-suppressive miRNAs, and inhibiting MALAT1 could restore miRNA activity, offering a multi-target therapeutic approach [41-43].

In principle, the complex roles of ncRNAs in cancer biology underscore their potential as biomarkers for diagnosis and as targets for innovative therapies. Future advancements in delivery technologies and combination strategies are likely to unlock their full therapeutic potential.

3. Current Epigenetic Therapies in Cancer Treatment

Epigenetic therapies target the reversible modifications that regulate gene expression, offering a promising approach for cancer treatment. These therapies focus on correcting aberrant epigenetic patterns, such as hypermethylation or hypoacetylation, which disrupt normal gene expression and contribute to tumorigenesis [9]. Unlike conventional therapies that directly target the DNA sequence or proteins, epigenetic therapies modulate the chromatin environment to reactivate silenced tumor suppressor genes or inhibit oncogenic pathways [44,45]. The U.S. Food and Drug Administration (FDA) has approved several epigenetic drugs (summarized in Table 2), primarily for hematologic malignancies, including DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis). These agents highlight the clinical potential of reprogramming the cancer epigenome [46,47]. At present, histone deacetylase inhibitors represent the only approved epigenetic therapies in clinical use that target histone proteins. Nevertheless, significant research efforts are focused on developing selective inhibitors aimed at other enzymes involved in histone modification. Figure 2 provides an overview of the various epigenetic drug targets.

Table 2 FDA-Approved Epigenetic Drugs in Cancer Therapy

Drug	Class	Mechanism of Action	Approved Indications	Challenges
Azacitidine	DNA Methyltransferase (DNMT) Inhibitor	Inhibits DNMT enzymes, leading to hypomethylation of DNA and reactivation of silenced tumor suppressor genes.	Myelodysplastic Syndrome (MDS), Acute Myeloid Leukemia (AML)	Limited efficacy in solid tumors, risk of DNA damage, and off-target toxicity
Decitabine	DNA Methyltransferase (DNMT) Inhibitor	Similar to azacitidine, incorporates into DNA, causing DNMT degradation and gene reactivation.	Myelodysplastic Syndrome (MDS), Acute Myeloid Leukemia (AML)	Poor stability and limited activity against non-hematologic malignancies
Vorinostat	Histone Deacetylase (HDAC) Inhibitor	Inhibits HDAC activity, leading to increased acetylation of histones and reactivation of silenced genes.	Cutaneous T-Cell Lymphoma (CTCL)	Reversible effects, systemic toxicity, and moderate activity in solid tumors
Romidepsin	Histone Deacetylase (HDAC) Inhibitor	Promotes accumulation of acetylated histones, inducing apoptosis in cancer cells.	Peripheral T-Cell Lymphoma (PTCL), Cutaneous T-Cell Lymphoma (CTCL)	Severe side effects like fatigue and immunosuppression, limiting long-term use
Tazemetostat	EZH2 Inhibitor	Targets EZH2, a histone methyltransferase, reducing trimethylation at H3K27 and reactivating tumor suppressor genes.	Follicular Lymphoma, Epithelioid Sarcoma	Limited efficacy as a monotherapy and potential resistance development

3.1. FDA-Approved Epigenetic Drugs

3.1.1. DNMT Inhibitors

DNA methyltransferase inhibitors, such as azacitidine (Vidaza) and decitabine (Dacogen), are nucleoside analogs that inhibit DNA methylation by irreversibly binding to DNMT enzymes. These drugs are primarily used to treat myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). DNMTis work by incorporating into DNA during replication, trapping DNMT enzymes and leading to their degradation. This results in demethylation of CpG islands, reactivating silenced tumor suppressor genes and restoring normal cell function [47].

Azacitidine was the first epigenetic drug approved by the FDA in 2004 for the treatment of MDS. It induces differentiation and apoptosis in cancer cells by reactivating genes involved in cell cycle regulation and apoptosis [48]. Decitabine, approved in 2006, shares a similar mechanism of action but is more DNA-specific, targeting hematopoietic progenitor cells to enhance its therapeutic efficacy [49]. Both drugs have shown improved survival rates and hematologic response in patients with high-risk MDS and AML [48,49].

Although DNMT inhibitors have demonstrated efficacy in hematologic malignancies, their application in solid tumors has been limited due to challenges in drug delivery and the complex tumor microenvironment [50]. Ongoing research is exploring combination therapies, such as DNMT inhibitors paired with immune checkpoint inhibitors or chemotherapy, to enhance their effectiveness in solid tumors.

3.1.2. HDAC Inhibitors

Histone deacetylase inhibitors (HDACis) target the enzymes responsible for removing acetyl groups from histones, leading to chromatin compaction and transcriptional repression. By inhibiting HDACs, these drugs promote a relaxed chromatin state, facilitating the reactivation of silenced genes and inducing cancer cell apoptosis, differentiation, and cell cycle arrest. Four HDAC inhibitors are FDA-approved, primarily for cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL) [49,51].

Vorinostat (Zolinza), approved in 2006, is a hydroxamic acid-based HDAC inhibitor used to treat CTCL. It disrupts oncogenic transcriptional programs by increasing acetylation of histones and non-histone proteins, thereby inhibiting tumor cell proliferation and angiogenesis [52,53]. Romidepsin (Istodax), approved in 2009, is a cyclic peptide HDAC inhibitor with potent activity against PTCL and CTCL. It induces apoptosis in cancer cells through the accumulation of acetylated histones, restoring tumor suppressor gene function [54].

Both vorinostat and romidepsin are effective as monotherapies, but their side effects, such as fatigue, gastrointestinal disturbances, and hematologic toxicity, limit their widespread use. Combination therapies with other epigenetic drugs, chemotherapy, or immunotherapy are being investigated to maximize their therapeutic potential while minimizing adverse effects [51-54].

Epigenetic therapies represent a transformative approach in oncology, leveraging the reversibility of epigenetic modifications to reprogram cancer cells. DNMT and HDAC inhibitors have paved the way for this class of drugs, offering hope for improved outcomes, particularly in hematologic cancers. Advances in drug design and combination strategies continue to expand the scope of these therapies, including their application to solid tumors and resistant cancer types.

3.2. Emerging Therapeutic Agents in Epigenetic Cancer Therapy

Bromodomain and extraterminal (BET) proteins and enhancer of zeste homolog 2 (EZH2) represent key epigenetic regulators that contribute to oncogenesis through dysregulated chromatin dynamics. Therapeutic agents targeting these proteins, including BET inhibitors and EZH2 inhibitors, are promising tools in cancer treatment. Their ability to reprogram the cancer epigenome and complement other therapies is increasingly explored in both preclinical and clinical settings [55].

BET inhibitors target bromodomains, which are critical epigenetic "reader" domains recognizing acetylated lysines on histone tails, facilitating the recruitment of transcriptional machinery to oncogene promoters and enhancers. Inhibitors such as JQ1 displace BET proteins like BRD4 from chromatin, thereby reducing oncogenic transcription [55,56]. According to researchers from the American Association for Cancer Research, BET inhibitors preferentially disrupt super-enhancer-associated oncogenes like MYC and BCL2, which are vital for cancer cell survival and proliferation (AACR, 2024) [57]. In a study by Reyes-Garau et al. [58], the efficacy of BET inhibitors was demonstrated in hematologic malignancies such as acute myeloid leukemia (AML), where they decreased the expression of survival genes. From the findings of Senapedis et al. [59], BET inhibitors also synergize with immune checkpoint inhibitors, enhancing T-cell-mediated antitumor responses, especially in MYC-driven solid tumors [59].

EZH2 inhibitors like tazemetostat are another class of emerging epigenetic therapies. EZH2, a catalytic subunit of the Polycomb Repressive Complex 2, silences tumor suppressor genes through trimethylation of histone H3 at lysine 27 (H3K27me3) [60]. Overexpression or mutations in EZH2 have been implicated in aggressive cancers, such as follicular lymphoma and certain sarcomas. According to P. A. Lazo [61], tazemetostat suppresses EZH2 activity and restores the expression of silenced tumor suppressor genes. In a separate study by Lue et al. [62], tazemetostat demonstrated notable efficacy in treating EZH2-mutant follicular lymphoma and showed potential in combinations with DNA methyltransferase inhibitors (DNMTis) to augment reactivation of tumor suppressor pathways. Furthermore, tazemetostat has been explored for solid tumors like prostate cancer, albeit with limited single-agent efficacy, highlighting the need for combination strategies [59-62].

These agents represent a significant advance in cancer therapy. While single-agent therapies highlight their biological impact, the integration of BET and EZH2 inhibitors into combinatorial regimens targeting multiple epigenetic and immune pathways is expected to overcome resistance mechanisms and expand therapeutic applicability in diverse cancer types. Their clinical success will hinge on continued exploration in well-designed trials to optimize efficacy and manage potential toxicity [60,62]

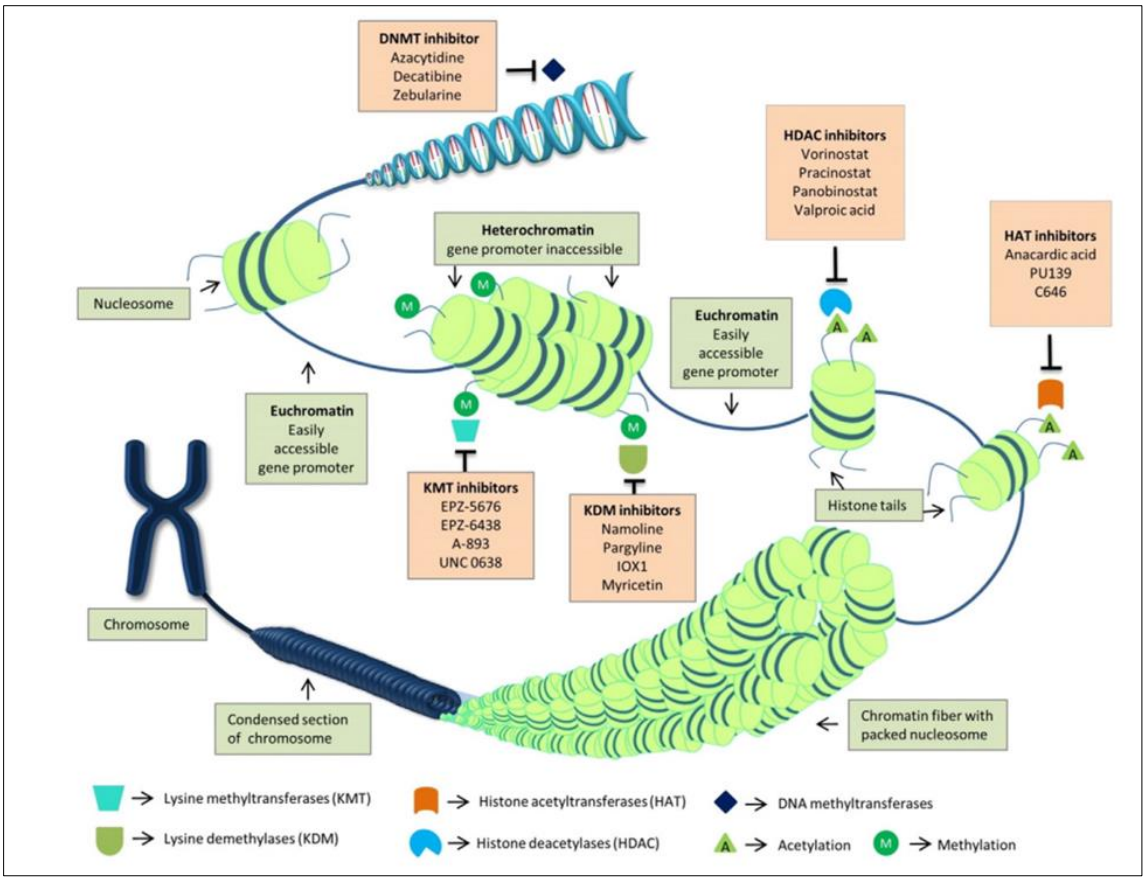

Figure 3 Schematic diagram showing the various epigenetic drug targets in cancer therapy, including DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis), KDM inhibitors, KMTninhibitors. Reproduced from Ref. [25] with permission, copyright, Elsevier 2017

3.3. Combination Therapies in Epigenetic Cancer Treatment

Epigenetic therapies offer a promising approach to overcoming the limitations of conventional cancer treatments. Their combination with immunotherapy and chemotherapy or targeted therapies enhances therapeutic outcomes by reprogramming the tumor microenvironment and improving immune recognition of cancer cells. These combinations (summarized in Table 3) aim to maximize treatment efficacy while minimizing resistance and toxicity.

Table 3 Combination Therapies with Epigenetic Drugs in Cancer Treatment

Epigenetic Drug	Combination Partner	Mechanism of Synergy	Cancer Type	Clinical/Preclinical Evidence
Azacitidine (DNMTi)	Anti-PD-1/PD-L1 Immune Checkpoint Inhibitors	Reactivates tumor suppressor genes and enhances immune cell infiltration into the tumor microenvironment.	Bladder Cancer, Melanoma	Combination improves efficacy of immune checkpoint inhibitors by increasing immune cell activity.
Vorinostat (HDACi)	Chemotherapy (e.g., Cisplatin, Carboplatin)	Reduces apoptotic threshold in cancer cells, making them more sensitive to DNA-damaging agents.	Non-Small Cell Lung Cancer (NSCLC)	Preclinical studies show enhanced cancer cell apoptosis and DNA repair disruption.
Tazemetostat (EZH2i)	Anti-CTLA-4 Antibodies	Prevents immune suppression by inhibiting regulatory T-cell (Treg)	Solid Tumors, including Melanoma	Demonstrated slower tumor progression and enhanced survival in animal models.

		conversion, increasing CD8+ cytotoxicity.		
Romidepsin (HDACi)	IL-2 (Immunotherapy)	Enhances the activation of immune-related genes and antigen presentation, amplifying immune response.	Hodgkin's Lymphoma	Increased survival and immune activation in clinical trials with HDAC inhibitors and IL-2.
Decitabine (DNMTi)	BET Inhibitors	Combined epigenetic modulation reactivates suppressed genes while reducing oncogene expression.	Acute Myeloid Leukemia (AML)	Enhanced differentiation and apoptosis in preclinical studies.

3.3.1. Epigenetic Drugs with Immunotherapy (e.g., Checkpoint Inhibitors)

Epigenetic modifications often lead to immune evasion in cancer cells by downregulating genes involved in antigen presentation and immune response pathways. Combining epigenetic drugs with immune checkpoint inhibitors (ICIs) has demonstrated significant potential in reversing these immune-resistant phenotypes. According to Xie et al. [63], inhibitors of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) increase the expression of tumor-associated antigens, enhancing the visibility of cancer cells to cytotoxic T cells. This reactivation improves the efficacy of checkpoint inhibitors targeting PD-1/PD-L1 and CTLA-4 pathways, particularly in tumors that previously exhibited resistance to ICIs [63].

From the findings of Goswami et al. [64], the combination of the EZH2 inhibitor tazemetostat with anti-CTLA-4 antibodies enhanced CD8+ T cell cytotoxicity and reduced regulatory T cell (Treg) activity in murine melanoma models. Additionally, the combination increased immune cell infiltration into the tumor microenvironment, slowed tumor progression, and improved responses to anti-PD-L1 therapy in preclinical models of bladder cancer. These results emphasize the immunotherapy-sensitizing effects of epigenetic agents, broadening their application beyond hematologic malignancies to solid tumors [63,64].

3.3.2. Synergy with Chemotherapy or Targeted Therapies

The integration of epigenetic drugs with chemotherapy or targeted therapies has shown promise in overcoming resistance and enhancing the efficacy of standard treatments. According to Duan et al. [65], epigenetic drugs can lower the apoptotic threshold of cancer cells, making them more susceptible to DNA-damaging agents like platinum-based chemotherapies. For instance, combining DNMT inhibitors such as azacitidine with chemotherapeutic agents like cisplatin significantly enhanced tumor cell death in preclinical studies [65].

In a separate study by Hontecillas-Prieto et al. [66], HDAC inhibitors demonstrated synergy with tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC), enhancing apoptosis and inhibiting metastatic pathways. The addition of HDAC inhibitors to TKIs also overcame resistance mechanisms mediated by epigenetic alterations. These findings underscore the value of epigenetic drugs in resensitizing cancer cells to therapies that would otherwise be ineffective.

Epigenetic drugs also augment the effects of targeted therapies by modulating chromatin states and transcriptional programs. For example, the combination of bromodomain and extraterminal domain (BET) inhibitors with targeted agents like PI3K inhibitors has shown promise in preclinical models of breast cancer by simultaneously targeting multiple oncogenic pathways [65,66].

4. Opportunities in Epigenetic Therapy Development

Epigenetic therapy is a dynamic and rapidly evolving field, offering immense potential to refine cancer treatment strategies. The reversibility of epigenetic modifications and their role in tumor progression have positioned them as promising targets for therapeutic intervention. Recent advancements in biomarker discovery and non-invasive monitoring tools have further enhanced the prospects of tailoring epigenetic therapies to individual patients, optimizing therapeutic outcomes while minimizing side effects. A key focus in this area is the use of epigenetic biomarkers and liquid biopsy technologies, which together facilitate real-time tracking of disease progression and treatment response.

4.1. Biomarker-Driven Personalization

4.1.1. Role of Epigenetic Biomarkers in Predicting Therapeutic Response

Epigenetic biomarkers, including DNA methylation patterns, histone modifications, and non-coding RNAs, are critical tools for predicting therapeutic responses and stratifying patients for personalized treatments. These biomarkers can identify subsets of patients more likely to benefit from specific epigenetic therapies. According to Van Vlodrop et al. [67], DNA hypermethylation in tumor suppressor genes is commonly observed in cancer, and its reversal using DNMT inhibitors can serve as a predictive indicator of therapeutic efficacy. According to their research findings, CpG island hypermethylation plays a critical role in cancer by silencing tumor suppressor and DNA repair genes, which disrupts normal gene regulation and contributes to tumor development. The study underscores that the specific location of hypermethylation within promoter regions, particularly near transcription start sites (TSS), is crucial for determining its biological and clinical relevance. Furthermore, these methylation patterns hold promise as biomarkers for cancer detection, prognosis, and predicting therapy responses. The authors also critique existing methylation analysis techniques, advocating for more precise approaches to identify and validate clinically significant hypermethylation markers [67].

Moreover, histone modification patterns and non-coding RNAs are gaining attention for their role in predicting therapy response. As highlighted by Yamagishi et al. [68], the presence of specific histone marks, such as H3K27me3, correlates with resistance or sensitivity to certain therapies. These insights have paved the way for the integration of biomarker-driven strategies in clinical trials, improving patient selection and enhancing treatment outcomes [68].

4.1.2. Liquid Biopsy for Tracking Epigenetic Changes in Real-Time

Liquid biopsy technologies represent a transformative approach in oncology, offering non-invasive and real-time monitoring of epigenetic changes in patients. Unlike traditional tissue biopsies, which are invasive and provide a static view of the tumor, liquid biopsies enable continuous assessment of tumor dynamics. According to a study made by Wang et al. [69], circulating tumor DNA (ctDNA) and extracellular vesicles (EVs) in blood samples can reveal critical epigenetic alterations, including DNA methylation and miRNA profiles. These biomarkers provide insights into tumor burden, treatment efficacy, and the emergence of resistance (See Figure 4) [69].

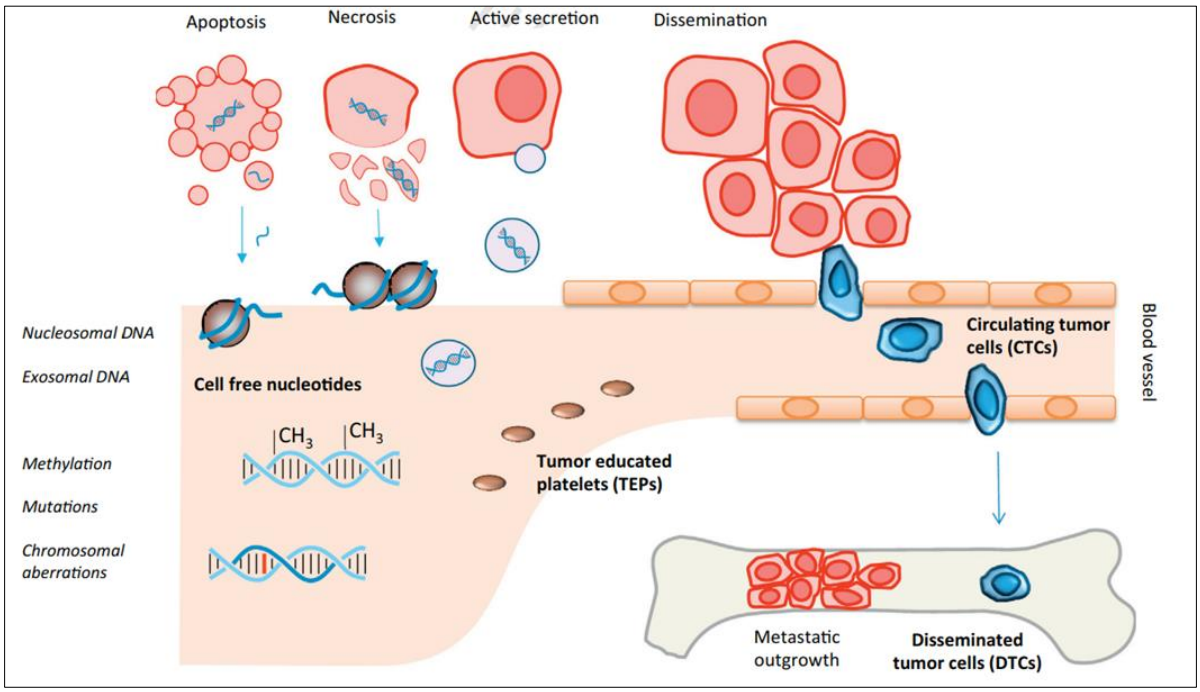

Figure 4 Schematic diagram of Liquid biopsy markers. These biomarkers provide insights into tumor burden, treatment efficacy, and the emergence of resistance. Reproduced from Ref. [71] with permission, copyright, Elsevier 2018

Advanced techniques such as droplet digital PCR (ddPCR) and methylation-specific PCR (MSP) have improved the sensitivity and specificity of detecting epigenetic changes in liquid biopsies. For example, according to Nell et al. [70]

and other researchers ddPCR allows for the detection of methylated DNA fragments in as many as 20,000 droplets, enhancing the precision of methylation analysis in cancers such as lung and colorectal cancer. By analyzing bisulfite-converted DNA, ddPCR can detect methylation levels as low as 0.5% in a given sample. This makes it particularly effective for studying methylation patterns in cancers like lung and colorectal cancer, as it provides robust sensitivity and reproducibility, even for rare or low-abundance methylation signals [70-72]. This level of detail enables oncologists to tailor treatment regimens dynamically based on the molecular evolution of the tumor, improving the overall success of epigenetic therapies.

Liquid biopsies are particularly valuable in detecting minimal residual disease (MRD) and monitoring for disease recurrence. As emphasized in a review by Honoré et al. [73], these techniques offer a comprehensive view of tumor heterogeneity, capturing genetic and epigenetic alterations across primary and metastatic sites. This capability not only informs treatment adjustments but also identifies patients at risk of relapse, enabling early intervention and better outcomes [70,73].

4.2. Technological Advances in Epigenetic Therapy Development

Technological advancements have been pivotal in expanding the scope of epigenetic therapy by offering precise tools to study tumor heterogeneity and manipulate the epigenome. Single-cell epigenomics and CRISPR-based epigenome editing are two groundbreaking technologies that have transformed our understanding of cancer biology and therapeutic development.

4.2.1. Single-Cell Epigenomics for Heterogeneity Studies

Single-cell epigenomics enables the study of epigenetic modifications at an unprecedented resolution, revealing the diversity of cellular states within tumors. Tumors are inherently heterogeneous, consisting of subpopulations of cells with distinct epigenetic profiles that contribute to therapeutic resistance and disease progression. Techniques like single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) allow researchers to map these variations comprehensively.

According to Hu et al [7], single-cell sequencing has been instrumental in identifying malignant subpopulations and their interactions with the tumor microenvironment. In their work, they emphasized the critical role of single-cell sequencing technologies in uncovering tumor heterogeneity through epigenetic mechanisms. scRNA-seq studies on pancreatic ductal adenocarcinoma revealed significant heterogeneity among malignant and stromal cells, including immune suppressive subsets, which are potential therapeutic targets. These technologies surpass bulk sequencing by providing detailed insights into chromatin accessibility, DNA methylation, and histone modifications at the individual cell level. This enables a deeper understanding of tumor progression, treatment resistance, and potential therapeutic targets. This technology has also been applied to study metastatic lung cancer, uncovering dynamic gene expression patterns associated with resistance and disease progression during targeted therapy [74,75].

Moreover, single-cell epigenomics provides insights into the interplay between tumor and immune cells. In studies of gastric cancer, single-cell analyses of cancer-associated fibroblasts (CAFs) highlighted their diverse roles in shaping the immune microenvironment and influencing therapeutic outcomes. By distinguishing subpopulations like inflammatory CAFs and extracellular matrix CAFs, researchers have proposed novel therapeutic strategies targeting these cells to improve treatment efficacy [7, 76,77].

4.2.2. CRISPR-Based Tools for Precise Epigenome Editing

CRISPR technology has evolved beyond genome editing to enable precise modulation of the epigenome. CRISPR-based epigenome editing employs catalytically dead Cas9 (dCas9) fused to epigenetic modifiers to target specific loci without introducing permanent genetic changes. This approach allows for the reversible regulation of gene expression by altering chromatin states.

In a study carried out by Bhattacharjee et al. [78], the researchers reported that CRISPR-dCas9 systems fused with histone acetyltransferases or methyltransferases were used to activate or repress gene expression at targeted loci. This precise control is particularly useful for reactivating silenced tumor suppressor genes or inhibiting oncogenes in cancer cells. For instance, targeting promoter regions of the tumor suppressor gene *CDKN2A* led to significant reactivation and tumor suppression in preclinical models [78,79].

CRISPR-based tools also enable functional studies of epigenetic modifications, providing insights into their role in cancer progression. From the findings of Hu et al. [7], CRISPR systems have been combined with single-cell sequencing

technologies to explore epigenetic heterogeneity, enabling high-throughput screening of epigenetic regulators and their contributions to therapeutic resistance.

Beyond research, CRISPR-based epigenome editing holds promise for therapeutic applications. Early preclinical studies are exploring its potential to target specific epigenetic alterations in cancers with high heterogeneity. These applications could lead to innovative treatments that address the dynamic and adaptive nature of cancer epigenomes.

4.3. Targeting the Tumor Microenvironment

The tumor microenvironment (TME) consists of a dynamic and complex interplay between cancer cells, stromal components, and immune cells. Epigenetic modifications within these non-cancerous cells significantly influence tumor progression and therapeutic resistance. By targeting the epigenetic landscape of stromal and immune cells, it is possible to remodel the TME, potentially improving therapeutic efficacy.

4.3.1. Influence of Epigenetic Changes in Stromal and Immune Cells

Epigenetic alterations within stromal and immune cells contribute to the development of a pro-tumorigenic TME. According to Xie et al. [63], DNA methylation and histone modifications in tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) reprogram these cells into phenotypes that promote tumor growth and immune evasion. For example, TAMs with hypermethylated promoter regions of pro-inflammatory cytokines exhibit reduced anti-tumor responses [80,81]. Similarly, CAFs undergo epigenetic reprogramming, enhancing their matrix-secreting properties and promoting angiogenesis and tumor invasion [81].

In a study by Yang et al. [82], histone acetylation in T-regulatory (Treg) cells within the TME was found to suppress the anti-tumor immune response, further highlighting the role of epigenetic regulation in immune suppression. Targeting these modifications can reinvigorate immune cells, restoring their ability to combat tumor cells effectively [82].

4.3.2. Potential for Remodeling the Tumor Microenvironment Through Epigenetics

The reversibility of epigenetic changes offers opportunities to reprogram the TME into an anti-tumorigenic state. Epigenetic drugs, such as histone deacetylase inhibitors (HDACis) and DNA methyltransferase inhibitors (DNMTis), have shown promise in this regard. According to Dai et al. [83] and Dan et al. [84], DNMTis can enhance the expression of tumor antigens, making cancer cells more visible to immune cells. Furthermore, HDACis can reduce the suppressive activity of CAFs and TAMs, thereby improving immune cell infiltration and activation within tumors [83,84].

A separate study by Fang et al. [84] demonstrated that targeting histone modifications in endothelial cells of the TME can inhibit angiogenesis, a process crucial for tumor growth and metastasis. This highlights the broader potential of epigenetic therapies in disrupting tumor-supportive stromal networks and promoting an anti-tumorigenic microenvironment [84].

5. Challenges in Epigenetic Therapy Development and Clinical Application

While epigenetic therapies have demonstrated significant promise in cancer treatment, their development and clinical application face notable challenges. These include off-target effects, toxicity, and variability in patient response. Many of these limitations stem from the lack of specificity of current epigenetic inhibitors, which can affect non-target genes and pathways, leading to adverse side effects. Moreover, the complex pharmacokinetics and limited stability of some drugs further hinder their broader use [85]. Addressing these challenges (summarized in Table 4) is essential for realizing the full potential of epigenetic therapies in precision medicine.

Table 4 Challenges in Epigenetic Therapy Development and Clinical Application

Challenge	Description	Examples	Potential Solutions
Off-Target Effects	Non-specific interactions with non-cancerous genes, causing unintended gene activation or silencing.	HDAC inhibitors impacting non-histone proteins, leading to systemic toxicity.	Development of isoform-specific inhibitors and advanced delivery systems.
Delivery Inefficiencies	Difficulty in effectively delivering drugs to solid tumors or specific tissues.	DNMT inhibitors like decitabine showing reduced efficacy in solid tumors.	Use of nanotechnology-based delivery systems for targeted distribution
Therapeutic Resistance	Cancer cells adapting to evade treatment through mutations or compensatory pathways.	Activation of alternative oncogenic pathways in response to DNMT and HDAC inhibitors.	Combination therapies targeting multiple pathways to prevent adaptive resistance.
Toxicity	Adverse systemic effects due to the lack of precise targeting mechanisms.	Myelosuppression and gastrointestinal toxicity caused by DNMT inhibitors.	Biomarker-driven patient selection and reduced dosing strategies
Tumor Heterogeneity	Genetic and epigenetic diversity within tumors, reducing therapy effectiveness.	Varied responses to HDAC inhibitors in different cancer subtypes.	Personalized therapy based on comprehensive tumor profiling.
High Cost and Accessibility	Development and clinical application costs limit access to epigenetic drugs for broader patient populations.	Limited availability of advanced therapies like Tazemetostat in low-resource settings.	Policies to support affordable pricing and research subsidies for novel therapies.
Lack of Biomarkers	Difficulty in identifying predictive biomarkers to guide therapy.	Absence of reliable markers to predict response to DNMT and HDAC inhibitors.	Advanced research to discover and validate biomarkers for patient stratification

5.1. Off-Target Effects and Toxicity

5.1.1. Non-specificity of Current Inhibitors and Potential Side Effects

One of the primary concerns with epigenetic drugs is their lack of specificity, which can lead to unintended modulation of non-targeted genes and pathways. This is particularly evident with first-generation DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis). According to a review by Karagiannis et al. [28], these inhibitors often target multiple DNMT or HDAC isoforms, leading to widespread epigenetic changes that can disrupt normal cellular processes. For example, the use of azacitidine and decitabine, both DNMTis, has been associated with significant toxicity in organs such as the liver and heart due to their broad activity profiles [86,87].

In a separate study by Xiao et al. [88], HDAC inhibitors such as vorinostat and romidepsin demonstrated efficacy in treating cutaneous T-cell lymphoma but caused off-target acetylation in non-cancerous tissues, resulting in side effects like fatigue, gastrointestinal disturbances, and myelosuppression. This highlights the need for more selective inhibitors that can target specific isoforms or chromatin regions to minimize systemic toxicity [88].

Additionally, the inability to precisely target epigenetic modifications within the tumor microenvironment exacerbates these challenges. Epigenetic drugs can inadvertently affect normal stromal and immune cells, potentially leading to immunosuppressive effects or altering the tumor microenvironment in ways that favor tumor growth. As reported in a study by Cheng et al. [89], broad histone modifications induced by HDAC inhibitors can suppress the activity of tumor-infiltrating lymphocytes, counteracting the intended therapeutic benefits [89].

Strategies to Address Non-specificity and Toxicity

To overcome these issues, second-generation epigenetic drugs are being developed with improved specificity and pharmacokinetics. For instance, guadecitabine (SGI-110), a next-generation DNMT inhibitor, has shown reduced toxicity by selectively targeting cancer cells while sparing normal tissues. Similarly, the design of isoform-specific HDAC

inhibitors aims to limit off-target effects. According to research by Sykes et al. [90], combining these drugs with targeted delivery systems, such as nanoparticles, has further enhanced their specificity and reduced systemic toxicity.

Future approaches involve integrating advanced drug delivery platforms, such as liposomal encapsulation or antibody-drug conjugates, to ensure that epigenetic therapies are localized to the tumor site. These methods hold promise for mitigating off-target effects and improving the overall tolerability of epigenetic treatments [91,92].

5.2. Epigenetic Plasticity and Resistance

Epigenetic plasticity refers to the dynamic and reversible nature of epigenetic changes, enabling cancer cells to adapt to environmental pressures, including therapeutic interventions. This plasticity contributes significantly to drug resistance, as tumor cells exploit epigenetic reprogramming to survive and thrive under treatment-induced stress. According to a review by Ilango et al. [15], mechanisms such as DNA methylation, histone modifications, and chromatin remodeling allow tumor cells to undergo phenotypic switching, altering gene expression without permanent genetic mutations. This adaptability fosters cellular heterogeneity and the emergence of resistant subpopulations [15].

For instance, the phenomenon of "drug-tolerant persister cells" has been observed in cancers treated with targeted therapies. These cells enter a transient, drug-tolerant state mediated by epigenetic alterations such as histone deacetylation or methylation [93]. In a study by Sharma et al. [94], HDAC inhibitors reversed this tolerance, suggesting a key role for epigenetic mechanisms in transient resistance. Similarly, research on EGFR-mutant lung cancer demonstrated that resistant cells displayed altered transcriptional profiles driven by epigenetic regulators, contributing to therapeutic failure [93,94].

The reversibility of epigenetic modifications presents both challenges and opportunities. While this trait enables rapid adaptation, it also offers a potential therapeutic target. Epigenetic drugs like DNMT and HDAC inhibitors can reprogram resistant cells, sensitizing them to conventional treatments. However, as highlighted by Xiao et al. [88], the widespread and nonspecific effects of these agents may inadvertently promote secondary resistance or toxicity, underscoring the need for more precise approaches [88,94].

5.3. Delivery Challenges in Epigenetic Therapy

The development and application of epigenetic therapies for solid tumors are often hindered by delivery challenges and the inherent instability of these drugs. Solid tumors, characterized by their dense extracellular matrix, irregular vasculature, and hypoxic microenvironments, pose significant barriers to the effective penetration and distribution of epigenetic drugs [95]. Additionally, many epigenetic agents have poor pharmacokinetic profiles, including low bioavailability, rapid metabolism, and systemic toxicity, which further limit their clinical utility.

One of the critical challenges in treating solid tumors is ensuring adequate drug delivery to the tumor's hypoxic and dense regions. According to Vitorakis et al. [96], the hypoxic microenvironment within solid tumors not only fosters resistance to therapies but also influences the epigenetic landscape, necessitating precise delivery of epigenetic agents to these areas. The inefficiency of traditional drug delivery systems results in suboptimal therapeutic concentrations at the tumor site, contributing to limited efficacy in clinical trials [95,96].

Furthermore, the stability of epigenetic drugs, such as DNMT inhibitors (e.g., azacitidine) and HDAC inhibitors (e.g., vorinostat), is a major concern. These agents are often chemically unstable and susceptible to degradation in the bloodstream, leading to reduced activity before they reach the target site [97]. According to Nunes et al. [98], first-generation DNMT inhibitors rapidly degrade due to enzymatic hydrolysis, requiring high doses that increase systemic toxicity. This challenge has spurred the development of second-generation epigenetic drugs, such as guadecitabine, which feature improved stability and sustained release, but their application remains limited by delivery inefficiencies [96-98].

To address these issues, advanced drug delivery systems are being explored. Nanotechnology-based platforms, such as nanoparticles and liposomes, offer promising solutions by protecting epigenetic drugs from degradation, enhancing tumor-specific targeting, and improving bioavailability. In the study by Xiao et al. [88] discussed earlier, nanoparticle encapsulation of DNMT inhibitors demonstrated enhanced stability and selective delivery to tumor tissues, significantly reducing off-target effects and toxicity.

Another approach involves conjugating epigenetic drugs with tumor-penetrating peptides or antibodies to enhance their specificity and penetration into solid tumors. For instance, targeting ligands that recognize overexpressed

receptors in cancer cells have been used to improve the precision of drug delivery while minimizing systemic exposure [99,100].

5.4. Regulatory and Financial Barriers in Epigenetic Therapy Development

The development of epigenetic therapies faces significant challenges due to the high costs associated with drug discovery, clinical trials, and market approval. Epigenetic drugs require extensive preclinical research and rigorous testing to demonstrate safety and efficacy. According to Frontiers in Pharmacology, these costs are compounded by the complexity of epigenetic mechanisms, which demand innovative, often resource-intensive technologies for drug design and validation. This drives up the financial burden on pharmaceutical companies and limits accessibility for smaller research groups [101].

The regulatory landscape for epigenetic therapies is equally challenging. Due to their novel mechanisms, epigenetic drugs must meet stringent requirements to prove that they can safely target specific pathways without causing off-target effects. As noted by Rolland et al. [102], many epigenetic drugs are required to undergo prolonged clinical trial phases to address concerns about their systemic impact, further delaying approval and inflating costs.

The financial burden is exacerbated by the need for personalized approaches in epigenetic therapy. Tailoring treatments to individual epigenomic profiles necessitates advanced diagnostic tools and stratified trial designs, which increase trial complexity and associated expenses [103,104]. This can discourage investment in developing therapies for rare cancers or those requiring significant personalization.

To address these barriers, public-private partnerships and funding from non-governmental organizations have been suggested as ways to alleviate financial constraints. Additionally, the integration of advanced computational models to predict therapeutic outcomes may streamline regulatory processes and reduce trial costs [103].

6. Future Directions and Innovations

Table 5 Future Innovations in Epigenetic Cancer Therapy

Innovation	Description	Examples	Potential Impact
Personalized Epigenetic Therapy	Tailoring treatments based on the patient's unique epigenetic profile and tumor characteristics.	Use of DNA methylation and histone modification patterns to select therapies.	Enhances treatment specificity, reduces adverse effects, and improves clinical outcomes.
AI-Driven Therapeutics	Leveraging artificial intelligence to analyze data and design optimized epigenetic therapies.	AI models identifying new drug targets (e.g., PD-L1) and simulating molecular dynamics.	Accelerates drug discovery, reduces costs, and refines personalized treatment plans.
Combination with Immunotherapy	Integrating epigenetic drugs with immune checkpoint inhibitors to overcome immune evasion.	Combining EZH2 inhibitors with anti-PD-1 therapies.	Improves immune responses, reduces resistance, and enhances efficacy against solid tumors.
Liquid Biopsy for Epigenomics	Utilizing non-invasive methods to detect epigenetic biomarkers in blood samples.	Detection of circulating methylated DNA or histones.	Enables early detection, real-time monitoring, and adjustment of therapy.
Cancer Vaccines	Designing personalized vaccines targeting epigenetically altered antigens.	Vaccines based on tumor-specific methylation patterns.	Enhances preventive strategies and therapeutic interventions for high-risk individuals.
Integration of Multi-Omics Data	Combining epigenetic, genomic and proteomic data to gain holistic insights into tumor biology.	Use of tools like CRISPR and ChIP-seq to study complex interactions.	Provides comprehensive understanding and precise therapeutic strategies.

The evolving field of epigenetics is entering a transformative phase with the development of next-generation therapeutics and their integration into combination treatment strategies. While traditional epigenetic drugs have established a foothold in oncology, recent advancements aim to overcome limitations such as off-target effects, toxicity, and resistance. Emerging technologies focus on designing selective inhibitors, multi-target agents, and incorporating epigenetic approaches into broader therapeutic frameworks like nanomedicine, RNA-based therapies, and personalized vaccines. These innovations promise to revolutionize the landscape of cancer treatment by improving specificity, efficacy, and patient outcomes [105,106].

6.1. Next-Generation Epigenetic Therapeutics

The advent of next-generation epigenetic drugs emphasizes specificity and the ability to target multiple pathways. Selective inhibitors are being developed to minimize off-target effects while precisely modulating specific enzymes. According to Frontiers in Pharmacology, highly specific inhibitors for bromodomains and histone deacetylases are under investigation to improve therapeutic outcomes while reducing systemic toxicity. For example, novel BET inhibitors have demonstrated the ability to disrupt oncogenic transcription selectively in hematologic and solid tumors [107]. In addition, multi-target agents aim to address the complexity of cancer epigenetics by simultaneously modulating multiple pathways involved in tumor progression. From the findings of Schröder et al. [108], dual-function inhibitors that target both DNMT and HDAC pathways have shown promise in preclinical models of lung and breast cancers. These agents not only reprogram tumor cells but also enhance the immune system's ability to recognize and destroy cancer cells.

6.2. Integration with Other Therapies

Combining epigenetic drugs with other therapeutic modalities has shown potential to enhance efficacy. RNA-based drugs, such as small interfering RNAs (siRNAs) and messenger RNA (mRNA) therapies, offer a complementary approach by modulating gene expression. In a study by Sahafnejad et al. [109], combining DNMT inhibitors with siRNAs targeting oncogenic pathways achieved greater tumor suppression in models of pancreatic cancer.

Nanomedicine plays a pivotal role in overcoming delivery challenges and improving drug stability. Nanoparticles can encapsulate epigenetic drugs, protecting them from enzymatic degradation while delivering them directly to the tumor site [110,111].

Personalized vaccines, designed to target tumor-specific antigens, can be amplified by epigenetic therapies that enhance antigen presentation. From the findings of Liu et al. [112], DNMT inhibitors boost the expression of tumor antigens and MHC class I molecules, improving the efficacy of cancer vaccines in clinical trials for melanoma and lung cancers.

6.3. Emerging Research Areas

Three-dimensional (3D) epigenomics investigates the spatial organization of chromatin in the nucleus, shedding light on the complex interactions between distant genomic regions. This field has expanded the understanding of gene regulation in cancer by uncovering how chromatin looping and topologically associating domains (TADs) influence the accessibility of genes to transcriptional machinery. Aberrations in these 3D structures, such as altered enhancer-promoter interactions, can dysregulate oncogenes or silence tumor suppressor genes.

According to Deng et al. [113], advanced techniques like Hi-C and ChIA-PET are pivotal for mapping chromatin interactions in cancer cells, enabling the identification of novel regulatory elements. These technologies have revealed that disrupted chromatin loops often correlate with aggressive tumor phenotypes and therapeutic resistance. For example, enhancer hijacking—a process where enhancers are redirected to activate oncogenes—has been implicated in cancers like glioblastoma and prostate cancer [114,115].

In a separate study by De Riso and Cocozza [116], targeting 3D chromatin interactions with epigenetic drugs, such as bromodomain inhibitors, demonstrated significant potential in restoring normal regulatory networks. This highlights the therapeutic potential of addressing spatial genome organization as part of epigenetic therapy.

6.4. Potential of Leveraging Artificial Intelligence in Epigenomic Data Analysis

Artificial intelligence (AI), particularly machine learning (ML) and deep learning (DL), has emerged as a transformative tool in epigenomics. AI technologies facilitate the integration and interpretation of vast datasets generated by high-throughput sequencing methods like ChIP-seq, ATAC-seq, and Hi-C. These tools can identify patterns and relationships in epigenomic data that would be challenging to discern through traditional statistical methods [117,118].

AI-driven approaches have been instrumental in identifying biomarkers, predicting patient responses, and discovering novel therapeutic targets in cancer. For instance, ML algorithms have been used to classify tumor subtypes based on their epigenomic profiles, providing insights into personalized treatment strategies [119]. In another application, DL models have successfully predicted the effects of specific epigenetic modifications on gene expression, enhancing the understanding of cancer progression mechanisms [120].

From the findings of Feng et al. [121], integrating AI with 3D epigenomics allows for the modeling of complex chromatin interactions and the prediction of their functional consequences. This approach is particularly valuable for identifying non-coding regions of the genome with regulatory potential, broadening the scope of targetable regions in cancer therapies.

AI technologies are also being leveraged to streamline drug discovery by predicting the efficacy and safety of epigenetic compounds. For example, by simulating the impact of epigenetic drug combinations on tumor heterogeneity, researchers can prioritize candidates for clinical trials, potentially reducing development time and costs [120,121].

7. Conclusion

Epigenetic therapies represent a transformative frontier in cancer treatment, offering novel ways to address the complexities of tumor biology. This review has detailed the significant progress in understanding and targeting the mechanisms underlying epigenetic regulation, such as DNA methylation, histone modifications, and non-coding RNAs. Current advancements, including FDA-approved epigenetic drugs, emerging therapeutic agents, and combination strategies, highlight the growing potential of these therapies to overcome resistance, enhance treatment specificity, and improve patient outcomes. However, significant challenges remain, including off-target effects, delivery inefficiencies, and the financial and regulatory hurdles associated with developing these innovative therapies.

The opportunities within this field are vast, driven by advancements in technologies such as single-cell epigenomics and CRISPR-based editing tools, which are enabling a deeper understanding of tumor heterogeneity and epigenetic plasticity. Moreover, the integration of epigenetic therapies with other modalities like RNA-based drugs, nanomedicine, and personalized vaccines offers the potential to redefine multi-modal cancer treatment strategies. Despite this promise, the complexities of epigenetic regulation and its dynamic nature pose hurdles that require ongoing research and innovation to surmount.

Looking forward, epigenetic therapies hold immense promise to redefine the landscape of oncology. By addressing the underlying epigenetic alterations that drive cancer progression, these therapies not only offer potential for curative outcomes but also pave the way for more personalized and precise treatments. Continued research is essential to overcome existing challenges, refine therapeutic approaches, and translate experimental findings into successful clinical applications. The future of epigenetics in cancer treatment lies in the collaborative efforts of researchers, clinicians, and industry stakeholders to harness its full potential for transformative impact.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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