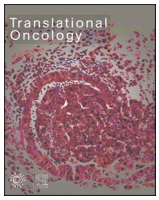
Translational Oncology 39 (2024) 101821

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon



Epigenetic modifications: Key players in cancer heterogeneity and drug resistance

Hana Q. Sadida [a], Alanoud Abdulla [a], Sara Al Marzooqi [a], Sheema Hashem [b], Muzafar A. Macha [c], Ammira S. Al-Shabeeb Akil [a,*], Ajaz A. Bhat [a,*]

[a] Laboratory of Precision Medicine in Diabetes, Obesity and Cancer, Department of Population Genetics, Sidra Medicine, Doha 26999, Qatar
[b] Laboratory of Genomic Medicine, Department of Population Genetics, Sidra Medicine, Doha 26999, Qatar
[c] Watson-Crick Centre for Molecular Medicine, Islamic University of Science and Technology, Jammu & Kashmir, India

ARTICLE INFO

Keywords:
Epigenetic modifications
Cancer heterogeneity
Drug resistance
DNA methylation
Histone modifications
Chromatin remodeling
Epigenetic therapies
Gene expression regulation

ABSTRACT

Cancer heterogeneity and drug resistance remain pivotal obstacles in effective cancer treatment and management. One major contributor to these challenges is epigenetic modifications - gene regulation that does not involve changes to the DNA sequence itself but significantly impacts gene expression. As we elucidate these phenomena, we underscore the pivotal role of epigenetic modifications in regulating gene expression, contributing to cellular diversity, and driving adaptive changes that can instigate therapeutic resistance. This review dissects essential epigenetic modifications - DNA methylation, histone modifications, and chromatin remodeling - illustrating their significant yet complex contributions to cancer biology. While these changes offer potential avenues for therapeutic intervention due to their reversible nature, the interplay of epigenetic and genetic changes in cancer cells presents unique challenges that must be addressed to harness their full potential. By critically analyzing the current research landscape, we identify knowledge gaps and propose future research directions, exploring the potential of epigenetic therapies and discussing the obstacles in translating these concepts into effective treatments. This comprehensive review aims to stimulate further research and aid in developing innovative, patient-centered cancer therapies. Understanding the role of epigenetic modifications in cancer heterogeneity and drug resistance is critical for scientific advancement and paves the way towards improving patient outcomes in the fight against this formidable disease.

Abbreviations

CircRNA	Circular RNA
CRC	Colorectal cancer
DNMT	DNA methyltransferase
LIF	Leukemia inhibitory factor
LncRNA	Long non-coding RNA
MiRNA	MicroRNA
NcRNA	Non-coding RNA
NK	Natural Killer cell
PiRNA	PIWI-interacting RNA
SiRNA	Small interfering RNA
TME	Tumor microenvironment
DNMT	DNA methyltransferase
MDR1	Multidrug resistance 1
HDACs	Histone deacetylases
PRC2	Polycomb repressive complex 2
NEAT1	Nuclear paraspeckle assembly transcript 1

Introduction

Epigenetic modifications have emerged as key players in the unfathomable complexities of cancer biology, driving an astounding degree of heterogeneity and leading to the frustrating challenge of drug resistance [1,2]. As the scientific community works tirelessly to unravel the mysteries of cancer, it is increasingly clear that understanding cancer at the molecular and cellular level is a critical endeavor that stands at the frontline of our battle against this relentless disease [3]. Cancer is not a single disease but a collection of diverse conditions, each with a distinct genetic and epigenetic landscape [4]. The vast heterogeneity seen in

* Corresponding authors at: Laboratory of Precision Medicine in Diabetes, Obesity and Cancer, Department of Population Genetics, Sidra Medicine, Doha 26999, Qatar
E-mail addresses: aakil@sidra.org (A.S.A.-S. Akil), abhat@sidra.org (A.A. Bhat).

https://doi.org/10.1016/j.tranon.2023.101821
Received 30 July 2023; Received in revised form 12 October 2023; Accepted 31 October 2023

cancer arises not just from genetic mutations but also from epigenetic changes that affect gene expression without altering the underlying DNA sequence [3]. This multifaceted diversity within a single tumor, known as intratumoral heterogeneity, poses a significant challenge for treatment, as different cellular sub-populations may respond variably or even resist therapeutic interventions [5].

Moreover, cancer cells exhibit inter-tumoral heterogeneity, i.e., differences between the primary tumor and its metastatic counterparts or between tumors of the same type in different patients [6]. Intrinsic or acquired drug resistance during treatment is a formidable obstacle in cancer management [7]. Unfortunately, the heterogeneity that makes cancer fascinating also makes it resilient, giving rise to subpopulations of cells that can survive therapy and promote recurrence [8]. Epigenetic modifications, including DNA methylation, histone modifications, and chromatin remodeling, have been recognized as significant contributors to cancer heterogeneity and drug resistance [9]. These modifications can dynamically regulate gene expression in response to external stimuli, fueling adaptive changes that allow cancer cells to survive and thrive even under therapeutic stress [10]. While genetic changes have a bigger effect on cancer heterogeneity, there is a far higher chance of spontaneous epigenetic alterations than genetic mutations, resulting in statistically higher epigenetic variability than genetic variability (PMID: 35018226). Importantly, unlike genetic changes, epigenetic modifications are reversible, thus presenting an opportunity for anti-cancer therapies that target epigenetic modulations [11–13]. In cancer biology, the understanding of epigenetics has been a breakthrough, revealing an intricate layer of regulation that can transform our understanding of the disease and offer new perspectives on therapeutic intervention [14]. However, the breadth and depth of epigenetic changes in cancer, their interactions with genetic alterations, and their influence on tumor heterogeneity and drug resistance are still not fully understood [15,16]. This review aims to delve into the role of epigenetic

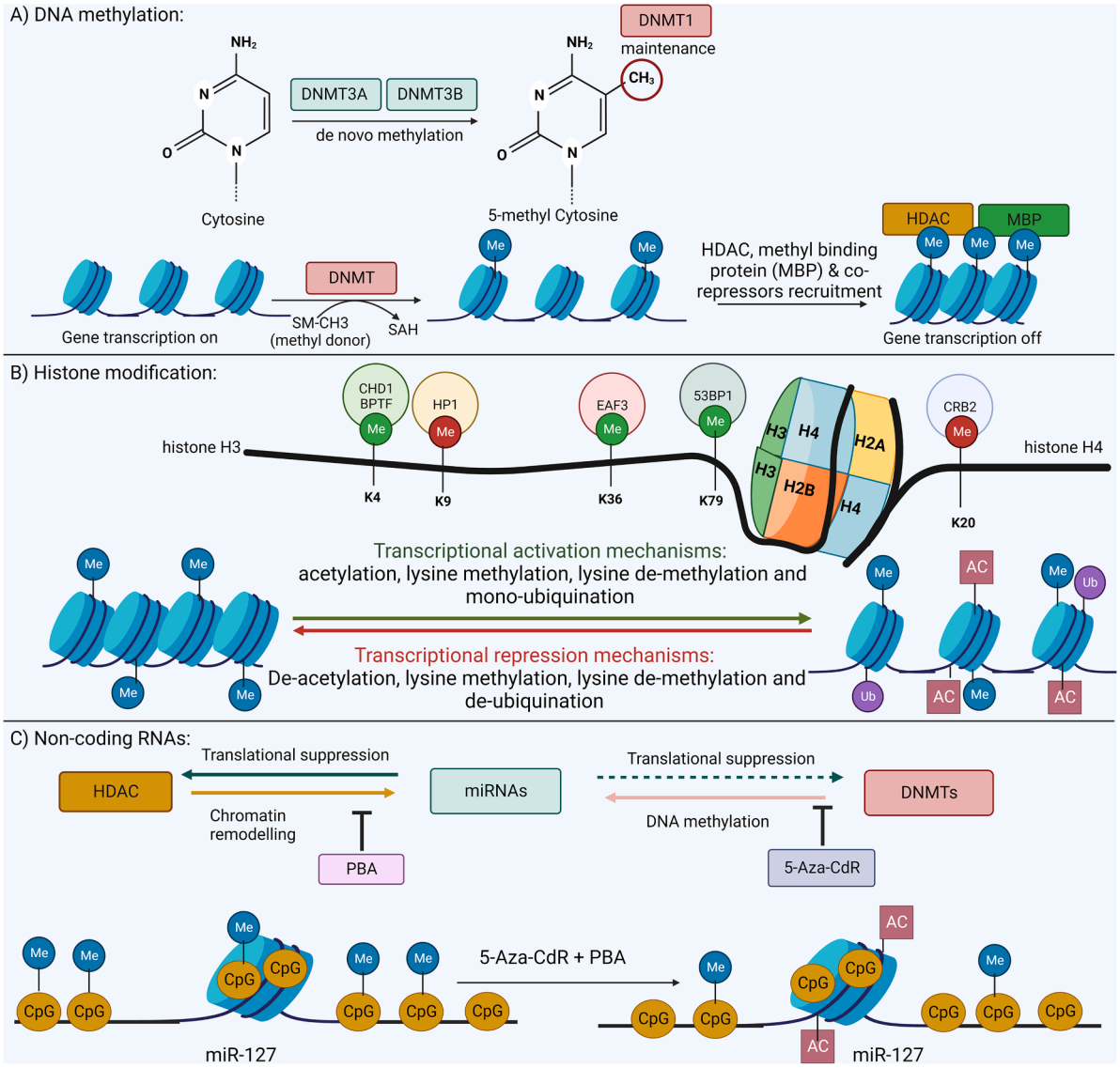


Fig. 1. Epigenetic mechanisms A) **DNA Methylation:** DNA methyltransferases (DNMTs) catalyze cytosine methylation by adding a methyl group. DNMT3A and DNMT3B perform de novo methylation, while DNMT1 maintains DNA methylation after DNA replication. Methylation, controlled by Dnmt and a methyl donor, targets CpG sites to add a methyl group to the DNA. Methylation and subsequent modifications by histone deacetylases (HDACs) and histone methyltransferases (HMTs) regulate gene expression. **B) Histone Modification:** Histone methylation occurs at the lysine residues in H3 and H4. Green and red methyl groups denote transcription activation and repression, respectively. Various proteins, including CHD1, BPTF, HP1, EAF3, 53BP1, and CRB2, participate in different processes, from transcription activation to DNA repair signaling. **C) Non-coding RNA:** DNA methylation and histone modifications control miRNAs expression, including miR-127. Methylation and deacetylation silence miR-127 in tumor cells, but treatment with 5′-Aza-CdR and PBA can reverse these modifications, resulting in miR-127 expression—miR-140 targets HDAC4 and DNMTs, indicating a two-way interaction between miRNAs and epigenetic enzymes.

modifications in cancer heterogeneity and drug resistance, providing a comprehensive overview of the current understanding of this important topic [17]. We aim to identify knowledge gaps and suggest potential future research directions. This review will cover the different types of epigenetic modifications and their roles in cancer biology, focusing on their contributions to intratumoral and inter-tumoral heterogeneity and drug resistance. Moreover, we will discuss the potential of epigenetic therapies to overcome drug resistance and the challenges involved in translating these findings into clinical practice [18,19]. We hope this review will stimulate further research in this rapidly evolving field and contribute to developing more effective strategies for managing cancer. Ultimately, the pursuit of understanding the role of epigenetic modifications in cancer heterogeneity and drug resistance is a mission to improve the lives of countless patients worldwide battling this formidable disease.

Epigenetic mechanisms in cancer

Epigenetic modification of DNA is a part of the control system within the cell that manages gene expression and silencing [20]. This is mainly through controlling the chromosomal structure rather than affecting the DNA sequence. Thus, the DNA sequence remains unchanged; however, the chromosomal substructure changes due to chemical modification in the chromosome. This, in turn, decides if the gene will be expressed and, subsequently, if the protein will be produced [21]. Epigenetic modification can be passed to the daughter cells maintaining the changes. Various types of epigenetic modifications (Fig. 1) work in different ways to affect gene expression, which will be discussed in this review [22].

DNA methylation

One of the ways to regulate chromatin compactness is DNA methylation. DNA methylation is the most studied epigenetic modification, which involves covalent DNA alteration by adding a methyl group that modifies the transcriptome and gene function. They are also heritable across mitosis [23]. Cytosine residues in CpG dinucleotides can be modified through methylation (addition of CH3) by the enzyme methyltransferase [24]. A methyl group from S-adenyl methionine gets transferred to the fifth carbon of a cytosine residue. This is the process that occurs in the early development of the embryo. In the first minutes of life when we are composed of single cells. All the epigenetic information is removed from the fertilized egg [25]. Through cell division, DNA methyltransferase (DNMT) adds methyl groups to some genes, specifically on their CpG islands which are common in the regulatory sequences such as promoter regions of genes. The methyl group then forms an obstacle to the transcription machinery preventing gene expression [26]. DNA methyltransferase (DNMT) enzymes such as DNMT3a, DNMT3b, and DNMT1 carry out the methylation process. During cell division, the epigenetic information gets transmitted to the newly formed cells by the enzyme DNMT1. Genes can only be expressed by removing the methyl group by the enzyme demethylase [27].

Histone Modifications

Histone is a protein whose positive charge allows it to combine with DNA. The basic repeating unit of chromatin is the nucleoside in which 146BP of DNA wraps around an octamer of core histone consisting of pairs of h3, h4, h2a, and h2b, condensing it into a protein. Histone proteins either have a side chain or a tail that is subjected to extensive covalent post-translational modifications (additions or removal of functional groups) [28]. Some modifications change charge density between histones and DNA, affecting the chromatin organization and transcription [29]. There are many different types of histone modification. Four of these modifications are well-studied and known for their significant impact on gene expression. Those are acetylation, methylation, phosphorylation, and ubiquitylation. These modifications affect gene expression by altering the ability to coil the DNA tightly, making some genes unreadable to the cells [30]. Changes in histone post-translational modification patterns are highly linked to cancer [31]. Histone modifications include citrullination, ubiquitination, ADP-ribosylation, deamination, formylation, O-GlcNAcylation, propionylation, butyrylation, crotonylation, and proline isomerization [32–35]. Modifications like these determine the structure, resulting in different DNA-binding proteins and chromatin compositions. When an acetyl group is added at different lysine residues located on the histone tail, basic charge neutralization compacts the chromatin [36]. Another study found that histone acetylation may regulate intracellular pH [37], confirmed by the results of many tumors with low intracellular pH and showing low histone acetylation levels, correlated to poor clinical outcomes. Histone acetylation is highly linked to active transcription, localized at enhancers and promoters [38]. Acetylation of H4 at lysine (K)16 is associated with cancer phenotype in different types of cancers [39] and has a potential prognostic value [40]. During hyperacetylation involving proto-oncogenes, activation of gene expression may occur; on the other hand, tumor suppressor hypoacetylation localizes to promoters, happening with DNA methylation, which leads to gene silencing [41]. The balance of acetylation is crucial because studies showed that histone acetyltransferase can act as an oncogene and tumor suppressor. Many of the mutations in histone acetyltransferase are detected frequently in different types of cancers. [38]. If histone deacetylases are altered, acetyl groups are removed by enzymes from the histone lysine residues, which have been seen in cancer [42]. Lysine and arginine residue methylation on histone tails indicate a complex chromatin modification compared to acetylation [31]. In addition, association with cancer has been indicated in low and high histone methylation levels [43].

Non-coding RNAs

Non-coding RNAs (ncRNAs) are RNA strands transcribed by RNA polymerase but not translated to a protein. These types of proteins either have a housekeeping or regulatory role. There are several classes of ncRNAs, such as small interfering RNA (siRNA), Long non-coding RNA (lncRNA), PIWI-interacting RNA (piRNA), and microRNA (miRNA). siRNAs and miRNAs are small ncRNAs that can bind to the 3′-untranslated region (UTR) of target mRNAs with assistance from other proteins, causing their degradation or inhibiting their translation [44]. In addition, it can recruit RNA-binding proteins that prevent histone deacetylation or transcription factors binding to the promoter region [45]. This causes gene silencing by repression of gene translation [46]. Transfer RNAs decode mRNA sequence into peptide or protein; it specifically recognizes three-nucleotide sequences of mRNAs and recruits amino acids in the correct order to ribosome. Ribosomal RNAs represent the most abundant RNA molecules in the cell. It also creates the ribosome framework, a macromolecular structure important to translate proteins [47]. Housekeeping RNAs are a requirement for regular cell function. Many housekeeping RNAs contain chemical changes added by a class of small nucleolar RNAs [48]. The long non-coding RNAs are another class of regulatory non-coding RNAs responsible for transcriptional activity. They are identified by a length of at least 200 nucleotides and have no potential for protein coding [49]. Studies have shown that long non-coding RNAs are important in epigenetic control of gene expression, promoter-specific gene regulation, imprinting and maintaining nuclear architecture [50–53]. Small and long regulatory non-coding RNAs are linked to different types of cancer [54]. Studies show the crucial functions of miRNAs in many types of cancers. miRNAs have high expression levels in cancer cells and enhance cancer development. Some of the miRNAs regulate cancer progression. miR-126 has high expression levels in breast [55] and colorectal cancers [56]. Overexpression of miR-126 in mouse hematopoietic stem progenitor cells led to B-cell leukemia. When miR-126 is overexpressed, it down-regulates p53 expression and its associated genes [57]. When miR-126 is suppressed,

apoptosis occurs, and B-ALL progression in xenograft mice is inhibited. miR-155 is found to be an oncogene in various types of cancers, such as colon, breast, lung, gastric and liver cancer [58–62]. It was detected that miR-155 was overexpressed in plexiform neurofibromas [49]. Overexpression of miR-155 increases proliferation and sphere formation of plexiform neurofibromas initiating cells. On the other hand, anti-miR-155 nucleic acid lowered the levels of tumors in the mouse spontaneous plexiform neurofibromas model. miR-215 is an oncogene overexpressed in glioblastoma by hypoxia [63].

Chromatin Remodeling

Chromatin is a mixture of DNA and proteins that comprise the chromosome. These chromatin fibers are coiled and condensed, forming a compact structure of chromosomes (heterochromatin) that can fit in the nucleus. This makes genes inaccessible for transcription. Thus, chromatin remodeling is required to change the chromatin structure to loosely packed euchromatin. Thus, allowing active gene transcription. Chromatin remodeling causes chromatin rearrangement from a condensed state to a transcriptionally accessible state, enabling transcription factor binding [64]. Chromatin remodeling can occur due to changing the nucleosome position by changes in the relative position of a few nucleosomes in a specific region or changes in the spacing of nucleosomes over a significant distance. Moreover, it can result in the removal of the histone octamers (histone eviction) or replacement with variant histones that are involved in active transcription (histone replacement) [65]. All these changes require nucleosome remodeling complexes such as SWI/SNF, ISWI, Mi2/Chd, and IN080. The common characteristic among these complexes is that they are ATP-dependent [66]. This DNA modification plays an essential role in transcriptional regulation and is vital for cell functioning; however, alterations within the mechanism may lead to many diseases, including cancer [15]. Tumor cells are known to have different methylomes compared to normal cells. Surprisingly, both hypomethylation and hypermethylation processes can be seen in cancer. Usually, low levels of methylated CpG content are typically detected, leading to genomic instability and sometimes activation [67]. The uncontrolled cell growth and subsequent cancer development may further allow cancer cells to evade drugs designed to inhibit these pathways.

The role of epigenetic modifications in cancer heterogeneity

Cancer cell subpopulations within the same tumor exhibit heterogeneity in their genotypes, phenotypes, and epigenetic profiles, accounting for differences in disease progression, malignancy profiles, and therapy response [68]. This intra-tumor heterogeneity trait is especially reported in cancer stem cells and is partly a consequence of epigenetic modulations [23]. There are many mechanisms involved in epigenetic modulation, but tumorigenesis is promoted when the alterations occur in genes regulating tumor growth, invasion, or metastasis [23]. The mechanisms in which epigenetic modifications give rise to cancer heterogeneity will be explored in the following section.

Impact of DNA methylation on cellular diversity

DNA methylation patterns have been observed to be aberrant in cancer, especially at the early stages of tumorigenesis, with widespread losses and promoter-focal gains in various regions [12]. Cancer patients with observed aberrant DNA methylation in the early stages have led to more malignant cancers, more aggressive cancer, and poorer outcomes [13,69]. Hypermethylation occurs in the CpG island region that silences critical tumor suppressor genes and DNA repair genes (such as P16NK4a and BRCA1 in breast cancer). At the same time, hypomethylation in the regions activates oncogenes, thus driving tumorigenesis [23,68,13].

Aberrant DNA methylation patterns in cancer contribute to cellular/histological heterogeneity and phenotypes diversity within tumor subpopulations by promoting the formation of subpopulations of cancer cells with distinct molecular/gene expression makeup and phenotypic feature as well as different cell differentiation states [12,13,23]. DNA methylation alterations are cell-specific and distinctive, and it is maintained due to the heritability nature of this mechanism [12]. The heterogeneous methylation patterns drive the clonal selection of a single tumor as they impart a selective advantage in tumor-initiating subpopulations during tumor evolution. The new clones that arise then divide into subclones with distinct features and methylation, increasing intra-tumour heterogeneity and plasticity – two important cancer hallmarks [12,13,23,68].

Additionally, DNA hypermethylation can also disrupt the balance between the active and repressed histone marks of stem-cell-related genes (H3K4me3, H3K27me3) by binding to their cancer-specific promoter and blocking transcription factors from binding, consequently inducing an abnormally silenced state of the gene and chromosome instability [12,70]. As a result, subclones/these diverse cancer cell subpopulations emerge that possess more stem-cell-like characteristics with self-renewal ability (called cancer stem cells) and fewer differentiation traits [12,71]. These cancer stem cells are key drivers of cellular diversity and intratumor heterogeneity that drive cancer progression [72]. Dysregulations in the methylation patterns in NANOG, a cancer stem cell marker, disrupt the switch between cancer stem cells and non-cancer stem cells [72].

Unlike genetic mutations, DNA methylation is reversible; thus, DNA hypermethylation observed in cancer patients can be targeted by using demethylating agents, bringing down the methylation levels to a normal range and transforming cancer cells into a more normal state by inducing differentiation.

Influence of histone modifications and chromatin remodeling on tumor microenvironment

The role of epigenetic alterations in cancer development and malignancy is well researched; however, their role in creating a favourable tumour microenvironment for cancer growth is often overlooked [73,74]. The tumor microenvironment (TME) consists of a network of cancer cells, surrounding normal cells, an extracellular matrix, and signaling molecules critical in promoting cancer growth and metastatic tendency [75]. Cells within TME, such as immune cells and fibroblasts, can also be subjected to epigenetic alterations, primarily by histone modifications and chromatin remodeling [74].

Chromatin forms the scaffold that packages the entire genome; thus, changes within chromatin modeling complexes (e.g., nucleosome position changes through nucleosome sliding, ejection, or histone eviction) would directly influence the cell phenotype [68]. Additionally, histone modifications, such as acetylation, methylation, and ubiquitination, can influence the structure and function of chromatin accessibility critical for transcription, thereby affecting gene expression [30,68,76]. Cancer patients' genomic-wide aberrant and variable histone modifications lead to heterogeneity of chromatin accessibility in different tumor subpopulations, which in turn causes heterogeneity in the expression of genes involved in cell proliferation, differentiation, and survival causing TME heterogeneity [68,77].

For instance, aberrant histone acetylation and methylation in cancer have been reported to alter the transcription of cytokines, chemokines, and transcription factors involved in the function and differentiation of T-cell and tumor-associated macrophages, which are critical components of TME. In turn, this leads to TME immunosuppression [77,78]. In breast and colorectal cancer, there is an overexpression of SATB1 (special AT-rich sequence binding 1), a chromatin regulator gene that facilitates histone modifications and chromatin remodeling in genes/its respective binding motif. Consequently, there is an increase in the secretion of pro-tumorigenic cytokine IL-6 and the production of immunosuppressive factor Galectin that switches inflammatory anti-tumour dendritic cells into pro-tumour dendritic cells, thus leading

to immunosuppression that drives tumorigenesis [74]. Similarly, histone acetylation causes a decrease in NK-cell receptor NKG2D expression that impairs the anti-tumor response of NK cells through immune exhaustion [74].In populations of mouse excitatory neurons and kidney tubule cells, scATAC-seq data revealed that there is significant heterogeneity in chromatin accessibility that is correlated with the location of the cell in relation to the parent tissue, showing that TME induces changes in cells that result in epigenetic heterogeneity [68]. Moreover, histone modifications also serve a vital role in the ability of TME to adapt to changes in the microenvironment (e.g., nutrient availability and oxygen levels) by regulating expressions of genes involved in cell survival and proliferation in hypoxic conditions of TME [68,79]. In multiple myeloma, abnormal chromatin remodeling occurs due to crosstalk with the microenvironment, resulting in chromatin activating the expression levels of key molecular pathway genes such as p53 [73].

Regulatory roles of Non-coding RNAs in cancer heterogeneity

Aberrant expression of ncRNAs from epigenetic mechanism disruptions, including miRNAs, lncRNAs, and circRNAs, play a critical role in regulating gene expression through complex formation correlated with carcinogenesis, metastasis, and TME remodeling [78,80]. Cancer elevates ncRNA expression, which is highly heterogeneous [81]. Alterations in ncRNAs can be context-dependent, as they can act as tumor suppressors or promoters in different contexts; thus, their role in different cancer types or cells differs, giving rise to heterogeneity [78]. miRNAs can prevent translation and promote degradation through post-transcriptional binding to mRNA, thus are implicated in the regulation of cell proliferation, differentiation, apoptosis, and immune response [78,82]. Dysregulation of miRNA expression drives cancer development and gives rise to its heterogeneity due to its ability to regulate cancer stem cells' differentiation state and function [80]. They can act as both tumor suppressors or oncogenes, which allows them to regulate the expression of many key cancer cellular and molecular processes such as p53 and RAS [83]. MiR-31 in breast cancer targets regulators of key signaling proteins such as RAS and WNT, resulting in an elevated level of proliferation and stem cell renewal, thus driving heterogeneity [80]. Similarly, miRNA can influence intercellular communication between components of the TME that drives tumorigenesis [83]

LncRNAs can modulate gene expression at the transcriptional, post-transcriptional, and epigenetic levels [84]. In cancer, dysregulated lncRNA expressions affect gene expression of genes regulating cell proliferation, differentiation, angiogenesis, and apoptosis, as well as cancer invasion and metastasis through their interactions with DNA and mRNA [85]. For instance, lncRNA can alter histone methylation due to its interaction with histone modification enzymes [86]. It can also form a complex with RNA-binding protein that stabilizes and elevates oncogenes' translation level. LncRNAs can also remodel the TME by promoting immune escape and metastasis, thus creating an immunosuppressive microenvironment via changes in tumor metabolism [84,86]. Distinct immune heterogeneity within the TME is established from LncRNAs [85].

CircRNAs are generated by back-splicing exons, introns, or both, forming a covalently closed circular structure [87]. They regulate gene expression by acting as miRNA sponges, interacting with RNA-binding proteins, or regulating transcription and splicing, so their dysregulation in cancer leads to phenotype heterogeneity [88,89].

Mechanisms behind epigenetic heterogeneity and challenges in cancer therapy

Cancer presents significant challenges in developing effective therapies. A significant aspect of cancer epigenetics is the heterogeneity within the same tumor or between tumors, critically impacting the expression of genes pivotal to cancer progression, metastasis, and therapeutic resistance [90,91]. This phenomenon, known as intratumor heterogeneity (ITH), makes it difficult in delineating consistent therapeutic targets applicable to all patients afflicted with identical types of cancer and is responsible for therapeutic failure [90,91].

Interestingly, while epigenetic changes can potentially reverse drug resistance, they also play a part in fostering it. For example, hypermethylation of DNA repair genes can induce resistance to standard treatments like chemotherapy and radiation therapy. Unfortunately, Current DNA methylation inhibitors tend to trigger hypomethylation, exacerbating genomic instability [91]. Given the reversible nature of epigenetic ITH, cancer cells demonstrate remarkable adaptability to treatments by continually reshaping their epigenetic landscapes, making it challenging to develop long-lasting therapies [92]. Additionally, epigenetic heterogeneity exhibits greater dynamism compared to that genetic heterogeneity, enabling cancer cells to transition between various epigenetic states. This plasticity not only encourages the emergence of resistant subclones but also frequently undermines the longevity of disease remission following treatment [93].

Epigenetic diversity is also a crucial driver of clonal evolution, a process wherein subclones, distinguished by unique epigenetic profiles, emerge over time. These subclones, owing to their diverse drug sensitivities and phenotypic advantages, may evolve into more aggressive, resilient populations, potentially leading to treatment failure and disease relapse [4,90]. The targeted therapies designed to tackle specific cancer-driving genetic mutations don't always account for the role of ITH, resulting in a lack of durable responses [68].

Compounding these challenges are the stem-cell-like attributes observed in many cancer cells, including capacities for self-renewal and differentiation. These properties are often linked to therapy resistance and recurrent tumor formation, further complicating the treatment landscape [91].

The variability introduced by epigenetic heterogeneity also complicates the advancement of personalized medicine. Identifying patient-specific epigenetic alterations for targeted therapy necessitates comprehensive, sophisticated profiling—a task made more daunting by the dynamic and reversible nature of epigenetic modifications [90,94]. This variability extends to the reliability of epigenetic biomarkers for early cancer detection or prognosis, as effective markers in one scenario may fail in others due to divergent epigenetic profiles [95].

To counteract the challenges posed by ITH, combination therapies often target both genetic and epigenetic aberrations, aiming to eradicate or neutralize all subclones within a tumor—a strategy known as synthetic lethality [68,91]. However, the unpredictability introduced by epigenetic heterogeneity can result in unforeseen interactions between combined treatments, complicating the optimization of therapeutic protocols [90]. Furthermore, the fluidity of epigenetic marks demands continual monitoring and recalibration of treatment strategies, a process that is not only resource-intensive but also poses logistical challenges.

In conclusion, epigenetic heterogeneity plays a pivotal role in the complexity of cancer and poses significant challenges in therapy development. Understanding the mechanisms contributing to this diversity and translating this knowledge into effective therapeutic strategies remain paramount in improving cancer treatment outcomes. Continued research and innovation in epigenetic manipulation and personalized therapy are crucial in the ongoing battle against this multifarious disease.

Epigenetic modifications and drug resistance in cancer

Mechanisms linking epigenetic alterations and drug resistance

Epigenetic modifications are pivotal in mediating drug resistance in cancer cells by inducing changes in gene expression profiles without alterations in the DNA sequence (Table 1). Key mechanisms include DNA methylation, histone modifications, and non-coding RNA-mediated regulations (Fig. 2).

Table 1
Epigenetic Modifications Associated with Drug Resistance in Cancer

Epigenetic Modification	Mechanism	Role in Drug Resistance	references
DNA Methylation	Hypermethylation of CpG islands in Multiple C2 domains transmembrane protein 1 (MCTP1), leading to gene silencing	In esophageal cancer cell line, drug sensitivity and resistance are decreased through the inactivation of tumor suppressor genes and drug transporters.	[173]
Histone Modifications	KMT1A is a histone methyltransferase that alters methylation patterns	It targets H3K9 histone, which affects drug targets, drug metabolism enzymes, and drug transporters, contributing to drug resistance in colorectal and lung cancer	[29]
Non-coding RNA	Dysregulation of specific microRNAs and long non-coding RNAs	Overexpression of lncRNA HOX transcript antisense RNA in cancers such as liver and colorectal leads to cyclin-dependent kinase inhibitor 1 downregulation. It is the main target of p53 activity DNA damage, which leads to drug resistance.	[174]
Chromatin Remodeling	Dysregulated chromatin remodeling complexes. Glioblastoma stem cells undergo slow cycling with stem cells qualities which develops tumor propagation and drug resistance	Alters the accessibility of DNA to drugs, affects DNA repair mechanisms and modulates drug target accessibility, contributing to drug resistance	[175]

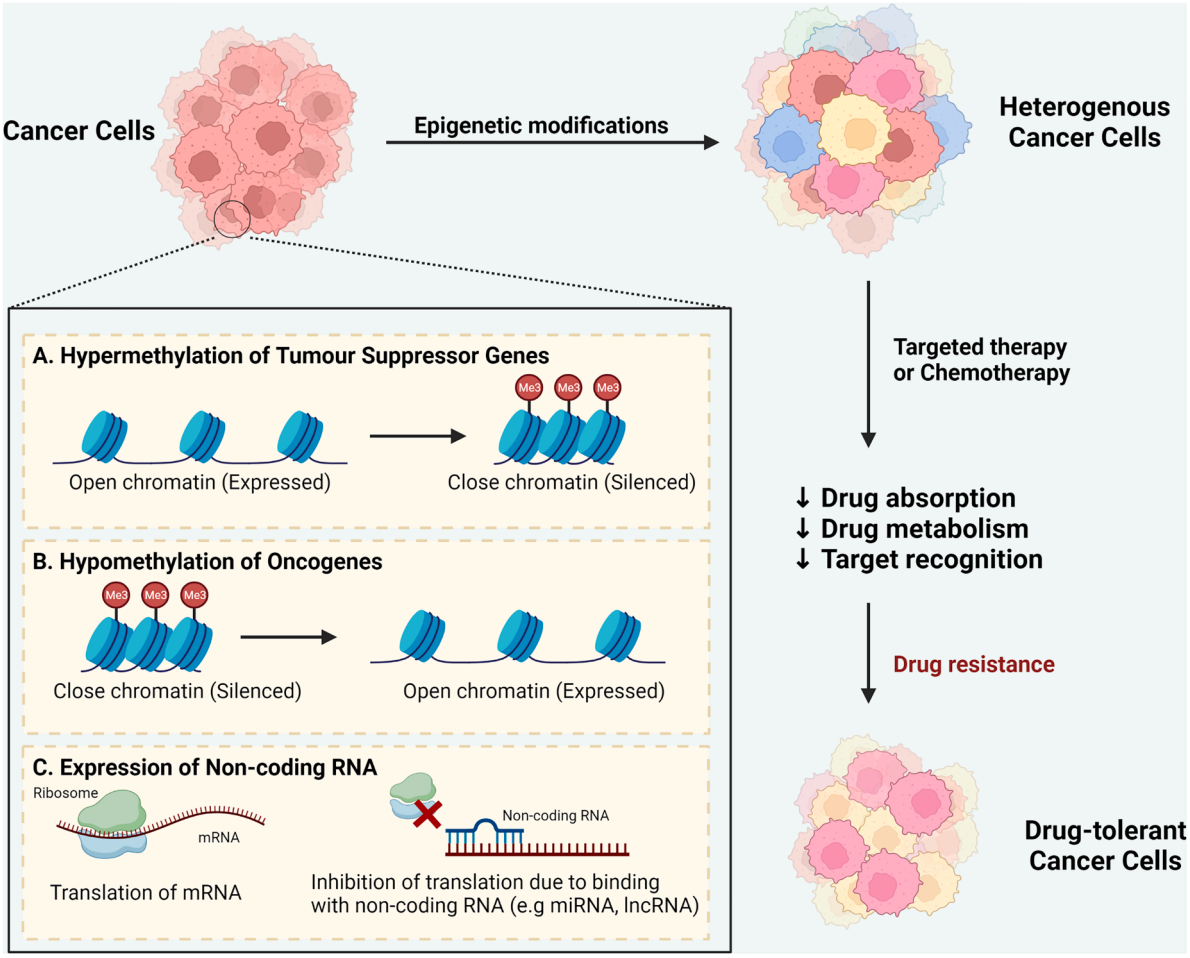


Fig. 2. Epigenetic Modulation in Cancer Drug Resistance: This illustration underscores the significant influence of epigenetic alterations in sculpting the drug resistance profile in cancer cells. Three key epigenetic phenomena are displayed: A) Hypermethylation of tumor suppressor genes, B) Hypomethylation of oncogenes, and C) Expression of non-coding RNAs. These modifications substantially affect the drug responsiveness and development of resistance by dynamically regulating the expression and functionality of genes critical to drug uptake, metabolism, and target interaction. Unraveling these intricacies paves the way for innovative strategies to circumvent drug resistance, enhancing cancer therapeutics' efficacy.

DNA methylation and drug resistance

DNA methylation, a fundamental epigenetic modification in mammalian cells, chiefly occurs at cytosine residues in CpG di-nucleotides. This modification typically leads to gene silencing when located in gene promoter regions. Aberrant DNA methylation patterns have been associated with many cancers and are considered a hallmark of the disease [96]. One connection between DNA methylation and drug resistance is through hypermethylation of CpG islands in the promoter regions of tumor suppressor genes [97]. Such an event can hinder the expression of these genes, leading to the unchecked growth of cancer cells and increased resistance to drugs that target these pathways (Table 1) [15]. Several studies have illustrated this mechanism, showing how promoter hypermethylation of genes such as MLH1, MGMT, and BRCA1 can induce resistance to different anti-cancer drugs [2,98]. In

non-small-cell lung cancer, hypermethylation of IGFBP3 leads to decreased sensitivity to cisplatin and hypermethylation of the MGMT gene has been reported to predict the efficacy of temozolomide in glioblastoma [99].

Conversely, global DNA hypomethylation, which often coincides with regional hypermethylation in cancer, can also be a route to drug resistance [100,101]. Hypomethylation may lead to increased oncogenes and gene expression in drug efflux. For instance, hypomethylation of the multidrug resistance 1 (MDR1) gene, which codes for the P-glycoprotein drug efflux pump, can enhance its expression, reducing intracellular drug concentrations and leading to drug resistance [98,2]. Hypomethylation also leads to the silencing of BRCA1, a major tumor suppressor gene implicated in breast cancer that ordinarily prevents cancer cell growth, leading to resistance to drugs like tamoxifen [102, 103].

Histone modifications and drug resistance

Histone modifications, another layer of epigenetic control, involve adding or removing functional groups to the histone proteins around which DNA is wound. Their modifications, which include methylation, acetylation, and phosphorylation, can significantly alter chromatin structure and gene expression, influencing various cellular processes, including drug response. Dysregulation of histone modifiers is often observed in cancer and can induce drug resistance [104]. For instance, histone deacetylase (HDAC) overexpression has been reported in various cancers and is associated with poor prognosis [105]. HDAC overexpression decreases histone acetylation levels, promoting a condensed, transcriptionally repressed chromatin state. This can silence the expression of pro-apoptotic genes, thus increasing cancer cell survival in the face of cytotoxic drugs (Table 1) [106,107,108]. Histone methylation is another important modification that influences drug response.

An example is the zeste homolog 2 (EZH2) enhancer, a histone methyltransferase that induces methylation of histone H3 at lysine 27 (H3K27me3), leading to transcriptional repression. Overexpression of EZH2 has been observed in multiple cancer types and linked to increased cell survival, proliferation, and drug resistance [1]. Targeting EZH2 has, therefore, been explored as a potential strategy to overcome drug resistance. Similarly, histone 3 methylation at lysine residues 9 or 27 can downregulate EGFR expression levels, which is amplified in colorectal cancer (CRC) and linked with transcriptional repression [109].

Non-coding RNA-mediated regulations and drug resistance

Non-coding RNAs (ncRNAs), which do not encode proteins but play vital regulatory roles, have emerged as crucial players in cancer drug resistance (Table 1) [110,19]. Numerous studies have demonstrated that dysregulated expression of miRNA, one of the classes of ncRNA, can modulate drug resistance by targeting key genes involved in apoptosis, cell cycle regulation, and drug metabolism and cause degradation/inhibition of translation [111,112]. For instance, miR-21 is frequently overexpressed in many cancers and has been shown to target several tumor suppressor genes, thus promoting cell survival and resistance to apoptosis-inducing drugs [113]. Meanwhile, long non-coding RNAs (lncRNA), which are longer than 200 nucleotides, can also influence drug resistance. They can interact with DNA, RNA, and proteins to modulate the chromatin state and gene expression. For instance, the lncRNA HOTAIR interacts with the polycomb repressive complex 2 (PRC2), guiding it to specific genomic loci to induce histone methylation and gene silencing. Upregulation of HOTAIR has been associated with drug resistance in various cancers [114]. Aberrant lncRNA expression has a major function in drug resistance in ovarian cancer therapy [115]. For instance, high levels of nuclear paraspeckle assembly transcript 1 (NEAT1) were detected in ovarian cancer cell lines and tumors. NEAT1 leads to paclitaxel drug resistance through upregulation of ZEB1 expression with the help of miR-194 [111].

Furthermore, some lncRNAs have been found to sponge miRNAs, thus indirectly affecting the expression of miRNA target genes and modulating drug resistance [116]. Moreover, lncRNAs contribute to chemotherapy resistance in CRC cells by regulating extracellular matrix proteins [117,118]. This could affect stromal cell phenotypes, such as cancer-associated fibroblasts, myeloid-derived suppressor cells, and tumor-associated macrophages, leading to TME reprogramming and enhanced CRC cell survival during drug treatment [119].

Strategies to overcome epigenetic drug resistance

Addressing the challenge of epigenetic drug resistance is critical in advancing cancer treatment. Various strategic approaches are being pursued, as highlighted by several recent studies. One major finding indicates that three members of the 48 drug-efflux pump ATP-binding cassette (ABC) transporter proteins, namely ABCB1, ABCC1, and ABCG2, are correlated with drug resistance in colorectal cancer (CRC) [119]. Overexpression of these transporters triggers an increase in drug efflux, resulting in resistance. For instance, the ABCB1 promoter's epigenetic regulation plays a significant role in drug transport in CRC cells, and changes in its methylation and histone H3 acetylation levels have been linked to altered pharmacokinetics of drugs like digoxin in vivo [120]. Another promising strategy involves targeting histone deacetylases (HDACs). Specifically, HDAC2 has shown elevated levels in CRC cell lines compared to normal colonic epithelial cells [16]. Silencing HDAC2 may lead to the downregulation of ABCB1 expression, thereby increasing the sensitivity of CRC cells to doxorubicin [16].

Additionally, using KDM4 inhibitors, an H3K9/36 tri demethylase has been demonstrated to reduce EGFR amplification, suggesting potential implications of epigenetic therapies in controlling EGFR copy-number in CRC [121]. Notably, the reversible nature of epigenetic modifications such as DNA methylation offers potential for therapeutic intervention. Drugs like Lethionine and ZCyd, acting as DNA methylation inhibitors, can be combined with traditional chemotherapy to enhance their efficacy [122].

Emerging evidence also supports the potential of non-coding RNAs (ncRNAs) as novel therapeutic targets for cancer therapy. For example, MRX34, a miR-34a mimic encapsulated within a liposomal nanoparticle, has shown promise in phase I clinical trials for patients with advanced solid tumors [123]. Additionally, miR-31-3p and miR-31-5p have emerged as potential predictive biomarkers for colorectal cancer in phase III clinical trials [112].

In a noteworthy computational study, a benzimidazole analog has demonstrated an inhibitory effect on the transformation of pri-miR-96 into the miR-96 oncogene, thereby increasing the expression of miR-96 target genes and promoting apoptosis in cancer cells [124]. Further exploration of benzimidazole resulted in the development of targaprimir-96, showing potential in reducing tumor burden in a triple-negative breast cancer xenograft mouse model [125].

In conclusion, understanding the intricate interplay between epigenetic modifications and drug resistance is critical in cancer treatment. These insights can facilitate the development of novel therapies, identify patients at higher risk of treatment failure, and guide the optimal choice of therapeutic strategies.

Benefits and limitations of epigenetic cancer therapies

Epigenetic therapies, targeting modifications that affect gene expression without altering the DNA sequence, have ushered in a new era in cancer treatment. Among these, DNMT inhibitors, ncRNAs, and histone modification inhibitors are particularly interesting. Each class of these agents, while showing promise, also brings unique challenges in clinical application.

DNMT inhibitors, such as 5-azacytidine and decitabine, target aberrant DNA methylation patterns in cancer cells [126]. These agents can reactivate tumor suppressor genes by demethylating promoter regions, inhibiting cancer growth, and promoting cellular differentiation - potentially attenuating the aggressive nature of cancer cells [92].

Additionally, they enhance the efficacy of other therapies, including chemotherapy and immunotherapy [92,127].

Despite their benefits, DNMT inhibitors' efficacy has limitations. They might induce remission or slow disease progression but not always eradicate cancer [128]. Their lack of specificity, leading to indiscriminate demethylation, manifests in cytotoxicity and adverse effects such as bone marrow suppression, fatigue, nausea, and increased infection susceptibility [92]. Resistance to these drugs can develop over time, possibly through re-methylation of DNA regions or other yet-unknown mechanisms. The optimization of dosing schedules remains an active area of research [127]. Furthermore, the high cost of some DNMT inhibitors may limit access for many patients. ncRNAs, encompassing microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), hold promise due to their specificity in modulating oncogenic pathways and their potential as diagnostic or prognostic biomarkers [129]. Their diverse mechanisms allow varied therapeutic approaches, and their low required dosages minimize side effects compared to traditional chemotherapy. Furthermore, they offer the possibility of combination therapies for enhanced effect [129].

However, several challenges impede their broad adoption. These include difficulties in achieving targeted delivery, risks of off-target effects, issues related to tumor heterogeneity, potential resistance, and a lack of extensive clinical validation as most ncRNA-based therapies are still experimental [130]. Continued research and clinical trials are needed to optimize their use, establish comprehensive safety and efficacy profiles.

Histone modification inhibitors, especially histone deacetylase inhibitors (HDACs), offer precise epigenetic targeting and have shown efficacy in certain cancers in slowing cancer growth [131]. They can alter histone acetylation levels, activate tumor suppressor genes, induce cell cycle arrest, suppress angiogenesis, and promote apoptosis, enhancing their therapeutic versatility [92]. They are more potent compared to other epigenetic drugs, albeit being more labile, and can produce therapeutical effect at low concentration with reduced side effects compared to chemotherapy.

Yet, these drugs have notable limitations. Their broad effects can induce off-target toxicity as they can influence both histone and non-histone proteins, and their success in clinical settings varies. Resistance can develop, possibly due to compensatory activities of other epigenetic modulators [91]. The intricate molecular interactions they target, coupled with tumor heterogeneity and the convoluted nature of epigenetic regulation, further complicate their application [132]. Additionally, not all cancers respond to these therapies equally, and their delivery to specific cells poses challenges [133].

In conclusion epigenetic therapies for cancer, encompassing DNMT inhibitors, ncRNAs, and histone modification inhibitors, offer groundbreaking avenues for treatment. However, their application is not without challenges, including side effects, development of resistance, issues with specificity, and variable efficacy among different cancer types. Future research must strive to optimize these treatments through precise targeting, dose refinement, resistance management, and comprehensive understanding of their roles in the multifaceted landscape of cancer epigenetics. As these therapies evolve, a nuanced, individualized approach will be paramount in maximizing their potential and minimizing their limitations.

Epigenetic therapies in clinical trials and beyond

In recent years, our increased understanding of epigenetic mechanisms deregulated in cancer has opened new vistas in therapeutic interventions. This knowledge offers a treasure trove of opportunities that can potentially reshape the landscape of cancer therapeutics. The intricate dance of epigenetic modifications stands central to our understanding of gene expression and cellular identity. This epigenetic choreography often goes awry in cancer, leading to pathological gene expressions that drive tumorigenesis. Our growing grasp of these epigenetic aberrations promises to transform therapeutic paradigms. Here, we delve deeper into the clinical and pre-clinical advancements in epigenetic therapeutics (Table 2).

Clinical trials of epigenetic therapies: a recap

Several therapeutic approaches rooted in epigenetic insights have made it to clinical trials:

DNMT inhibitors

Recent advancements in epigenetic research have elucidated the potential therapeutic value of DNA methyltransferase inhibitors (DNMTis) in oncology. Key agents in this category, such as azacitidine and decitabine, have garnered attention due to their demethylating properties and promising results in treating various malignancies (Table 2). A seminal study identified that decitabine facilitates the ubiquitination and subsequent degradation of all three DNMT variants. This degradation process is orchestrated through the TRAF6 pathway and culminates in protein degradation via a lysosome-dependent mechanism [134]. Both azacitidine and decitabine have exhibited significant efficacy as demethylating agents. Clinical trials and studies have underscored their potential in treating hematological malignancies and solid tumors [135,136]. In a paradigm that underscores the synergy of therapeutic agents, carboplatin-treated ovarian cancer patients displayed heightened drug sensitivity upon pre-treatment with azacitidine [137] or decitabine [138,139].In respiratory oncology, non–small cell lung carcinoma patients administered azacitidine witnessed enhanced efficacy in their anticancer therapy [140]. However, the therapeutic landscape is not uniformly promising. In phase II clinical trial involving 13 patients with advanced triple-negative breast cancer (TNBC), a combined regimen of azacitidine and the histone deacetylase inhibitor, entinostat, unfortunately, showed no clinical response [141]. In the realm of hematological malignancies, an intriguing combination of venetoclax with either decitabine or azacitidine was tested on elderly acute myeloid leukemia (AML) patients who were deemed unfit for conventional chemotherapy. The study's findings were optimistic, suggesting that the regimen was well-tolerated and posed a promising therapeutic strategy [142]. Given the evidence from these studies, it becomes paramount to delve deeper into the therapeutic potential of DNMTis, especially in solid tumors. A critical research direction would be to stratify patients and pinpoint subpopulations that would derive maximum benefit from DNA demethylating agents.

Table 2
Summary of Clinical Trials on Epigenetic Therapeutics

Drug Name	Cancer Type	Trial Phase	Key Findings	References
Azacitidine	AML & myelodysplastic syndromes	Phase III	Significant improvement in overall survival	[142,176, 177]
Decitabine	AML	Phase III	Complete remissions in 40% of patients	[142,178, 179]
Vorinostat	Cutaneous T-cell lymphoma	Phase II	Positive response in 30% of patients	[144,180]
Romidepsin	Cutaneous T-cell lymphoma	Phase II	35% response rate with manageable side effects	[152,181]
KDM4 Inhibitor	Advanced and metastatic solid tumors	Phase I	Demonstrated target engagement with some anti-tumor activity	[159]
MRX34	Advanced solid tumors	Phase II	Clinical benefit observed in multiple tumor types	[123]

HDAC inhibitors

Histone deacetylase inhibitors (HDACis) represent a promising class of epigenetic therapeutics. Notably, the U.S. Food and Drug Administration (FDA) has endorsed Vorinostat and Romidepsin for the treatment of advanced cutaneous T-cell lymphoma (CTCL), bolstering their clinical relevance (Table 2). Multiple phase II trials have provided evidence for Vorinostat's efficacy in CTCL, with observed response rates approximating 30% [143,144]. Intriguingly, these outcomes were juxtaposed against compounds like bexarotene and denileukin diftitox to gauge relative effectiveness [145]. While Vorinostat's therapeutic promise in CTCL is evident, its efficacy across other oncological indications has been mixed. Clinical endeavors aimed at elucidating its impact on diverse malignancies, such as lymphoma, breast, ovarian, prostate, colorectal, non-small-cell lung, head and neck, and glioblastoma, yielded suboptimal outcomes [146–151]. Romidepsin has not only been evaluated in CTCL but also in a gamut of other cancers. In a notable phase II trial focusing on CTCL, Romidepsin exhibited a robust response rate surpassing 30% - a pivotal result that precipitated its FDA approval for advanced CTCL [151]. Moreover, Romidepsin's therapeutic landscape extends to peripheral T-cell lymphoma, where it demonstrated a compelling response rate of nearly 40% among patients with refractory manifestations. This efficacy was accompanied by tolerable toxicities [152]. However, similar to Vorinostat, Romidepsin's foray into treating other malignancies like glioblastoma, small-cell lung cancer, prostate, colorectal, and renal cancers was met with limited clinical success [153–158]. In light of these findings, it's evident that while HDACis, particularly Vorinostat and Romidepsin, have showcased potential in hematological malignancies like CTCL, their universal applicability across a spectrum of solid tumors remains a topic of ongoing research.

KDM4 Inhibitor

The therapeutic targeting of lysine demethylase 4 (KDM4) has garnered considerable interest in the oncological research community, with TACH101 emerging as a pivotal compound. Distinctively, TACH101 is currently the only potent KDM4 inhibitor that has advanced to phase 1 clinical trials, marking a significant milestone in the field of epigenetic therapeutics (Table 2) [159]. The primary clinical application for TACH101 lies in the treatment of gastrointestinal cancers, particularly those marked by high microsatellite instability, such as metastatic colorectal cancers1. This decision to evaluate its efficacy in these specific cancer types is rooted in the molecular mechanisms by which TACH101 operates. TACH101 exerts its therapeutic effects by stymieing the proliferation of an array of cancer cell lines. Its broad-spectrum antiproliferative action also encompasses patient-derived organoid models, solidifying its potential applicability across diverse cancer types [160]. Delving into the in vivo landscape, TACH101 was subjected to rigorous testing across multiple cancer cell lines. An integral part of this evaluation involved the determination of the half-maximal inhibitory concentration (IC50), which provides insight into the compound's efficacy. TACH101 demonstrated pronounced potency against specific cancer cell lines, particularly human acute T-cell leukemia, triple-negative breast cancer, and esophageal squamous cell carcinoma. These cell lines exhibited exceptionally low IC50 values of 0.0027, 0.0035, and 0.0053 μmol/l respectively, indicating a high degree of sensitivity to TACH101 [160]. TACH101′s pre-clinical success was epitomized by its landmark efficacy in animal models. Remarkably, this compound curtailed tumor growth by a staggering 100%, propelling it into the phase 1 clinical trials in 2022 [159].

MRX34

The burgeoning field of microRNA-based therapies has witnessed the emergence of MRX34, a liposomal mimic of microRNA-34a (miR-34a). While miRNAs play intricate roles in gene regulation, their modulation holds immense therapeutic potential, especially in oncological contexts. The clinical exploration of MRX34 stands as testament to this (Table 2). A pioneering first-in-human Phase 1 study was embarked upon to evaluate MRX34′s efficacy in patients diagnosed with a spectrum of solid tumors [123]. The types of cancers under scrutiny in this trial encompassed melanoma (non-cutaneous forms excluding uveal), small cell lung cancer, triple-negative breast cancer, sarcoma, and cancers of the bladder, kidney, and ovary. The ability of MRX34 to effectively deliver miR-34a to tumors reinforces its potential as a therapeutic agent. Despite these promising observations, the therapeutic journey of MRX34 was not devoid of critical safety concerns. Alarmingly, the administration of MRX34 was linked to the unfortunate demise of four trial participants. Such a stark adverse outcome accentuates the imperatives of refining the drug's safety profile and warrants rigorous investigations to delineate the underlying causatives.The untoward incidents associated with MRX34 underscore the necessity for its thorough reevaluation. Future endeavors must prioritize a holistic assessment of its therapeutic and toxicological dimensions. With patient safety at the helm, there is an unequivocal need to optimize the drug formulation and perhaps reconfigure its dosing paradigms to circumvent such grave outcomes.

Pre-clinical studies

Beyond the existing therapies, innovative pre-clinical studies are looking to diversify and refine our epigenetic interventions:

Non-coding RNAs (ncRNAs) as therapeutics

Non-coding RNAs, primarily comprising microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have emerged as potent molecular entities in the epigenetic therapy landscape. Their diverse roles in cellular processes and disease etiology, especially in oncological contexts, have rendered them attractive therapeutic targets. Leveraging RNA-targeting therapeutics entails either inducing miRNA-like functionalities for restoring or depleting specific miRNA levels or inhibiting interactions between miRNAs and their respective targets [161]. Such interventions can recalibrate aberrant cellular processes implicated in diseases. A salient limitation associated with RNA therapeutics pertains to their intracellular delivery. Owing to their inherent negative charge, RNA molecules face barriers in crossing the cell membrane. To circumnavigate this hurdle, chemical modifications are indispensable, enhancing both the pharmacokinetics and pharmacodynamics of the therapeutic RNAs [162]. Merging RNA-based interventions with conventional treatment modalities like chemotherapy or radiotherapy paves the way for reducing the essential dosage of RNA therapeutics, thereby mitigating the potential immune response associated with high doses [163,164] and enhanced Sensitivity. siRNAs, a class of non-coding RNAs, have demonstrated proficiency in targeting genes pivotal for mitotic spindle assembly. This capability holds significance as it amplifies the sensitivity of certain cancer cells, like lung cancer cells, to specific drugs. For instance, a study centered on non-small-cell lung cancer illustrated the potential of siRNAs to drastically reduce (up to 1, 000-fold) the crucial dose of the drug paclitaxel in vivo [165]. The advent of non-coding RNA therapeutics signifies a paradigm shift in epigenetic therapy. As research progresses, it will be paramount to resolve the extant challenges and harness the full therapeutic potential of these molecular marvels.

Combining epigenetic modifiers with checkpoint Inhibitors

Recent advancements in cancer therapeutics highlight the potential of combining epigenetic modifiers, such as DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors, with immune checkpoint inhibitors (CPIs) [166]. This combination strategy primarily aims to reverse tumor immune evasion.Tumor cells can undergo epigenetic changes that downregulate the expression of tumor antigens and molecules involved in antigen presentation, making them less recognizable to the host immune system [167]. DNMT inhibitors can demethylate the DNA, leading to the re-expression of these silenced genes, while HDAC inhibitors can promote gene expression by enhancing the acetylation status of histones, thereby improving the

chromatin structure conducive for gene transcription [168]. By reverting these epigenetic modifications, previously 'invisible' or 'masked' tumors can become 'visible' to the immune system. In such a scenario, combining these epigenetic modifiers with CPIs, which act by blocking immune inhibitory pathways, could potentially amplify the immune system's ability to detect and attack the tumor cells [169]. Thus, combining DNMT or HDAC inhibitors with checkpoint inhibitors not only holds promise for enhancing the efficacy of immunotherapies [170] but also for expanding the range of patients who could benefit from them.

Unconventional epigenetic regulators

The field of epigenetic research is continuously evolving, with the identification of unconventional agents that exhibit potential as epigenetic modulators. Interestingly, certain compounds, traditionally not linked to epigenetics, are now under the research spotlight for their potential regulatory functions. A noteworthy example is Vitamin C. Vitamin C, known predominantly for its antioxidant properties, has recently garnered attention in the context of epigenetic research. Preliminary pre-clinical studies suggest that Vitamin C may play a role in modulating the epigenome. Its potential mechanism of action could involve the enhancement of the activity of Ten-Eleven Translocation (TET) enzymes, which are involved in the demethylation of DNA [171, 172]. By doing so, Vitamin C may influence gene expression patterns, potentially impacting cellular processes relevant to disease progression and therapeutic responses. Such discoveries emphasize the importance of broadening the search for epigenetic modulators, encompassing agents not traditionally linked to epigenetic regulation, and underline the potential complexity and interconnectivity of metabolic, nutritional, and epigenetic landscapes.

Conclusion and future perspectives

The study of epigenetics and its role in cancer heterogeneity and drug resistance offers promising yet challenging vistas for future research. However, the complexity and dynamism of the epigenetic landscape, intertwined with genetic alterations, present unique obstacles that currently limit our understanding of its intricate regulation in cancer.

One significant challenge lies in the technological limitations of current research methods. Epigenetic modifications are highly dynamic and context-dependent, requiring high-resolution, time-sensitive methods for accurate detection and analysis. Furthermore, given the heterogeneity inherent in cancer, single-cell epigenomics approaches are required to dissect the diverse cell populations within a tumor. Developing and refining these techniques will undoubtedly propel our understanding of the role of epigenetics in cancer.

In addition, the interplay between genetic and epigenetic alterations adds another layer of complexity. How these alterations interact and influence each other and collectively shape the tumor landscape and response to therapy is a significant question that remains largely unexplored. As we move forward, integrative multi-omics approaches will be critical to unravel these intricate networks.

Despite these challenges, the field of cancer epigenetics holds immense potential for clinical application, particularly in personalized medicine. Given the reversibility of epigenetic modifications, there is an opportunity to develop targeted therapies that can modify the cancer epigenome and overcome drug resistance. Additionally, specific epigenetic signatures could be used as predictive biomarkers to select the most effective treatment strategy for individual patients.

Several promising avenues of research are on the horizon. An important direction will be systematically characterizing the cancer epigenome across different cancer types, stages, and treatments. This will not only shed light on the role of epigenetics in cancer development and progression but also reveal potential epigenetic vulnerabilities that can be exploited for therapy.

Moreover, further research is needed to understand how the tumor microenvironment, including immune and stromal cells, influences the cancer epigenome and contributes to heterogeneity and drug resistance. Understanding these interactions could lead to novel combination therapies targeting both the cancer cells and their microenvironment.

Lastly, developing novel epigenetic drugs and their integration into current therapeutic regimens is critical for future research. Preclinical and clinical studies to evaluate these drugs' efficacy, safety, and resistance mechanisms will be crucial for their successful application.

In conclusion, while our understanding of the role of epigenetics in cancer heterogeneity and drug resistance is still in its infancy, the prospects for future research are exciting. With ongoing technological advancements and concerted research efforts, we are poised to gain unprecedented insights into the cancer epigenome, paving the way for personalized therapies and improved patient outcomes. Despite the challenges, the future of cancer epigenetics looks bright, promising a transformative impact on our fight against cancer.

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors consent to the publication

Availability of data

Not applicable

Author contribution statement

AAB and ASAA contributed to the concept and design. AAB, HQS, SAM, AA, and SH contributed to the manuscript writing. HQS, SAM, and AA generated tables and figures. MAM, AAB, and ASAA performed critical revision and editing of the scientific content.

All the authors meet the criteria for authorship. This manuscript is not under consideration for publication elsewhere. Every author is aware of, has agreed to this paper's content, and is listed as an author on the paper. All the authors declared no potential conflict of interest involved with this work.

Declaration of Competing Interest

All the authors declared no potential conflict of interest involved with this work.

Funding

This study was funded by Sidra Medicine Research Fund to Ajaz A. Bhat [SDR400105] and Ammira S. Al-Shabeeb Akil [SDR400175]).

Acknowledgments

The authors express their gratitude to the Sidra Medicine Research Branch for providing the funding necessary to carry out this study. Figures were created by using BioRender. Open Access funding is provided by the Qatar National Library.

References

[1] P.A. Jones, J.P. Issa, S. Baylin, Targeting the cancer epigenome for therapy, Nat. Rev. Genet. 17 (10) (2016) 630–641.
[2] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, Carcinogenesis 31 (1) (2010) 27–36.
[3] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.

[4] R.A. Burrell, et al., The causes and consequences of genetic heterogeneity in cancer evolution, Nature 501 (7467) (2013) 338–345.
[5] N. McGranahan, C. Swanton, Clonal heterogeneity and tumor evolution: past, present, and the future, Cell 168 (4) (2017) 613–628.
[6] A.A. Alizadeh, et al., Toward understanding and exploiting tumor heterogeneity, Nat. Med. 21 (8) (2015) 846–853.
[7] C. Holohan, et al., Cancer drug resistance: an evolving paradigm, Nat. Rev. Cancer 13 (10) (2013) 714–726.
[8] M.J. Hangauer, et al., Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition, Nature 551 (7679) (2017) 247–250.
[9] A.P. Feinberg, M.A. Koldobskiy, A. Gondor, Epigenetic modulators, modifiers and mediators in cancer aetiology and progression, Nat. Rev. Genet. 17 (5) (2016) 284–299.
[10] W.A. Flavahan, E. Gaskell, B.E. Bernstein, Epigenetic plasticity and the hallmarks of cancer, Science 357 (6348) (2017).
[11] P. Chi, C.D. Allis, G.G. Wang, Covalent histone modifications–miswritten, misinterpreted and mis-erased in human cancers, Nat. Rev. Cancer 10 (7) (2010) 457–469.
[12] H. Easwaran, H.C. Tsai, S.B. Baylin, Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance, Mol. Cell 54 (5) (2014) 716–727.
[13] Y.W. Ahmed, et al., Epigenetic tumor heterogeneity in the era of single-cell profiling with nanopore sequencing, Clin. Epigenetics 14 (1) (2022) 107.
[14] M.A. Dawson, T. Kouzarides, Cancer epigenetics: from mechanism to therapy, Cell 150 (1) (2012) 12–27.
[15] M. Esteller, Epigenetics in cancer, N. Engl. J. Med. 358 (11) (2008) 1148–1159.
[16] B. Haven, et al., Registered report: A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations, eLife 5 (2016).
[17] J.S. You, P.A. Jones, Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 22 (1) (2012) 9–20.
[18] M. Terranova-Barberio, S. Thomas, P.N. Munster, Epigenetic modifiers in immunotherapy: a focus on checkpoint inhibitors, Immunotherapy 8 (6) (2016) 705–719.
[19] Y. Chi, et al., Long non-coding RNA in the pathogenesis of cancers, Cells 8 (9) (2019).
[20] A. Portela, M. Esteller, Epigenetic modifications and human disease, Nat. Biotechnol. 28 (10) (2010) 1057–1068.
[21] J.P. Hamilton, Epigenetics: principles and practice, Dig. Dis. 29 (2) (2011) 130–135.
[22] D.E. Handy, R. Castro, J. Loscalzo, Epigenetic modifications: basic mechanisms and role in cardiovascular disease, Circulation 123 (19) (2011) 2145–2156.
[23] B. Carter, K. Zhao, The epigenetic basis of cellular heterogeneity, Nat. Rev. Genet. 22 (4) (2021) 235–250.
[24] L.D. Moore, T. Le, G. Fan, DNA methylation and its basic function, Neuropsychopharmacology 38 (1) (2013) 23–38.
[25] S. Canovas, et al., DNA methylation in embryo development: epigenetic impact of ART (Assisted Reproductive Technologies), Bioessays (11) (2017) 39.
[26] Z. Siegfried, I. Simon, DNA methylation and gene expression, Wiley Interdiscip. Rev. Syst. Biol. Med. 2 (3) (2010) 362–371.
[27] B. Alaskhar Alhamwe, et al., Histone modifications and their role in epigenetics of atopy and allergic diseases, Allergy Asthma Clin. Immunol. 14 (2018) 39.
[28] K. Luger, et al., Crystal structure of the nucleosome core particle at 2.8 A resolution, Nature 389 (6648) (1997) 251–260.
[29] J.E. Audia, R.M. Campbell, Histone modifications and cancer, Cold Spring Harb. Perspect. Biol. 8 (4) (2016), a019521.
[30] Y. Zhang, et al., Overview of histone modification, Adv. Exp. Med. Biol. 1283 (2021) 1–16.
[31] A.J. Bannister, T. Kouzarides, Regulation of chromatin by histone modifications, Cell Res. 21 (3) (2011) 381–395.
[32] C. Martin, Y. Zhang, Mechanisms of epigenetic inheritance, Curr. Opin. Cell Biol. 19 (3) (2007) 266–272.
[33] M. Tan, et al., Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification, Cell 146 (6) (2011) 1016–1028.
[34] N. Herranz, et al., Lysyl oxidase-like 2 deaminates lysine 4 in histone H3, Mol. Cell 46 (3) (2012) 369–376.
[35] R.Y. Tweedie-Cullen, et al., Identification of combinatorial patterns of post-translational modifications on individual histones in the mouse brain, PLoS One 7 (5) (2012) e36980.
[36] T. Kouzarides, Chromatin modifications and their function, Cell 128 (4) (2007) 693–705.
[37] M.A. McBrian, et al., Histone acetylation regulates intracellular pH, Mol. Cell 49 (2) (2013) 310–321.
[38] V. Di Cerbo, R. Schneider, Cancers with wrong HATs: the impact of acetylation, Brief. Funct. Genomic. 12 (3) (2013) 231–243.
[39] S. De Bakshi, S. Das, A. Sengupta, Mirizzi syndrome in a patient with carcinoma of the gallbladder, Br. J. Surg. 79 (4) (1992) 371–372.
[40] D.B. Seligson, et al., Global levels of histone modifications predict prognosis in different cancers, Am. J. Pathol. 174 (5) (2009) 1619–1628.
[41] S.B. Baylin, P.A. Jones, Epigenetic determinants of cancer, Cold Spring Harb. Perspect. Biol. 8 (9) (2016).
[42] B. Barneda-Zahonero, M. Parra, Histone deacetylases and cancer, Mol. Oncol. 6 (6) (2012) 579–589.
[43] M. Busslinger, A. Tarakhovsky, Epigenetic control of immunity, Cold Spring Harb. Perspect. Biol. 6 (6) (2014).
[44] M.U. Kaikkonen, M.T. Lam, C.K. Glass, Non-coding RNAs as regulators of gene expression and epigenetics, Cardiovasc. Res. 90 (3) (2011) 430–440.
[45] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (3) (2009) 155–159.
[46] J.X. Yang, R.H. Rastetter, D. Wilhelm, Non-coding RNAs: an introduction, Adv. Exp. Med. Biol. 886 (2016) 13–32.
[47] H. Yan, P. Bu, Non-coding RNA in cancer, Essays Biochem. 65 (4) (2021) 625–639.
[48] T. Kimura, [Non-coding Natural Antisense RNA: mechanisms of action in the regulation of target gene expression and its clinical implications], Yakugaku Zasshi 140 (5) (2020) 687–700.
[49] Y. Na, et al., MicroRNA-155 contributes to plexiform neurofibroma growth downstream of MEK, Oncogene 40 (5) (2021) 951–963.
[50] B. Jin, et al., Let-7 inhibits self-renewal of hepatocellular cancer stem-like cells through regulating the epithelial-mesenchymal transition and the Wnt signaling pathway, BMC Cancer 16 (1) (2016) 863.
[51] X.X. Li, et al., The role of let-7 and HMGA2 in the occurrence and development of lung cancer: a systematic review and meta-analysis, Eur. Rev. Med. Pharmacol. Sci. 22 (23) (2018) 8353–8366.
[52] P. Tristan-Ramos, et al., The tumor suppressor microRNA let-7 inhibits human LINE-1 retrotransposition, Nat. Commun. 11 (1) (2020) 5712.
[53] J.R. Kennerdell, N. Liu, N.M. Bonini, MiR-34 inhibits polycomb repressive complex 2 to modulate chaperone expression and promote healthy brain aging, Nat. Commun. 9 (1) (2018) 4188.
[54] J. Wang, et al., Long Non-coding RNA EPIC1 promotes cell proliferation and motility and drug resistance in glioma, Mol. Ther. Oncolytic. 17 (2020) 130–137.
[55] F. Li, Expression and correlation of miR-124 and miR-126 in breast cancer, Oncol. Lett. 17 (6) (2019) 5115–5119.
[56] F. Ebrahimi, et al., Deregulation of miR-126 expression in colorectal cancer pathogenesis and its clinical significance, Exp. Cell. Res. 339 (2) (2015) 333–341.
[57] S.R. Chen, et al., Research on miR-126 in glioma targeted regulation of PTEN/PI3K/Akt and MDM2-p53 pathways, Eur. Rev. Med. Pharmacol. Sci. 23 (8) (2019) 3461–3470.
[58] A.A. Al-Haidari, I. Syk, H. Thorlacius, MiR-155-5p positively regulates CCL17-induced colon cancer cell migration by targeting RhoA, Oncotarget 8 (9) (2017) 14887–14896.
[59] T. Luan, et al., Long non-coding RNA MIAT promotes breast cancer progression and functions as ceRNA to regulate DUSP7 expression by sponging miR-155-5p, Oncotarget 8 (44) (2017) 76153–76164.
[60] C. Shao, et al., The value of miR-155 as a biomarker for the diagnosis and prognosis of lung cancer: a systematic review with meta-analysis, BMC Cancer 19 (1) (2019) 1103.
[61] C. Prinz, D. Weber, MicroRNA (miR) dysregulation during Helicobacter pylori-induced gastric inflammation and cancer development: critical importance of miR-155, Oncotarget 11 (10) (2020) 894–904.
[62] Q. Yu, et al., miR-155-5p increases the sensitivity of liver cancer cells to adriamycin by regulating ATG5-mediated autophagy, Neoplasma 68 (1) (2021) 87–95.
[63] J. Hu, X.F. Wang, HIF-miR-215-KDM1B promotes glioma-initiating cell adaptation to hypoxia, Cell Cycle 15 (15) (2016) 1939–1940.
[64] C. Wu, Chromatin remodeling and the control of gene expression, J. Biol. Chem. 272 (45) (1997) 28171–28174.
[65] W. Kobayashi, H. Kurumizaka, Structural transition of the nucleosome during chromatin remodeling and transcription, Curr. Opin. Struct. Biol. 59 (2019) 107–114.
[66] A.A. Reyes, R.D. Marcum, Y. He, Structure and Function of Chromatin Remodelers, J. Mol. Biol. 433 (14) (2021), 166929.
[67] M. Kulis, M. Esteller, DNA methylation and cancer, Adv. Genet. 70 (2010) 27–56.
[68] M. Guo, et al., Epigenetic heterogeneity in cancer, Biomark. Res. 7 (2019) 23.
[69] Y. Kanai, Alterations of DNA methylation and clinicopathological diversity of human cancers, Pathol. Int. 58 (9) (2008) 544–558.
[70] A. Roy, et al., Resetting the epigenome: methylation dynamics in cancer stem cells, Front. Cell Dev. Biol. 10 (2022), 909424.
[71] Z. Mazloumi, et al., Effect of aberrant DNA methylation on cancer stem cell properties, Exp. Mol. Pathol. 125 (2022), 104757.
[72] F. Naz, et al., Cancer stem cells: a major culprit of intra-tumor heterogeneity, Am. J. Cancer Res. 11 (12) (2021) 5782–5811.
[73] C. Chakraborty, S. Mukherjee, Molecular crosstalk between chromatin remodeling and tumor microenvironment in multiple myeloma, Curr. Oncol. 29 (12) (2022) 9535–9549.
[74] I. Lodewijk, et al., Tackling tumor microenvironment through epigenetic tools to improve cancer immunotherapy, Clin. Epigenetic. 13 (1) (2021) 63.
[75] A.J. Wolpaw, C.V. Dang, Exploiting metabolic vulnerabilities of cancer with precision and accuracy, Trends Cell Biol. 28 (3) (2018) 201–212.
[76] Y. Yang, M. Zhang, Y. Wang, The roles of histone modifications in tumorigenesis and associated inhibitors in cancer therapy, J. Natl. Cancer Center 2 (4) (2022) 277–290.
[77] M. Hieda, N. Matsuura, H. Kimura, Histone modifications associated with cancer cell migration and invasion, Methods Mol. Biol. 1238 (2015) 301–317.
[78] J. Yang, et al., Epigenetic regulation in the tumor microenvironment: molecular mechanisms and therapeutic targets, Signal Transduct. Target Ther. 8 (1) (2023) 210.
[79] C. Yuan, et al., Crosstalk of histone and RNA modifications identified a stromal-activated subtype with poor survival and resistance to immunotherapy in gastric cancer, Front. Pharmacol. 13 (2022), 868830.
[80] F.J. Slack, A.M. Chinnaiyan, The Role of Non-coding RNAs in Oncology, Cell 179 (5) (2019) 1033–1055.

[81] Z. Zhao, et al., Individualized lncRNA differential expression profile reveals heterogeneity of breast cancer, Oncogene 40 (27) (2021) 4604–4614.
[82] K.B. Reddy, MicroRNA (miRNA) in cancer, Cancer Cell Int. 15 (2015) 38.
[83] B.M. Hussen, et al., MicroRNA: a signature for cancer progression, Biomed. Pharmacother. 138 (2021), 111528.
[84] J. Yang, et al., LncRNAs in tumor metabolic reprogramming and immune microenvironment remodeling, Cancer Lett. 543 (2022), 215798.
[85] Y.L. Liang, et al., A lncRNA signature associated with tumor immune heterogeneity predicts distant metastasis in locoregionally advanced nasopharyngeal carcinoma, Nat. Commun. 13 (1) (2022) 2996.
[86] Y.N. Pi, et al., Long Non-Coding RNAs in the tumor immune microenvironment: biological properties and therapeutic potential, Front. Immunol. 12 (2021), 697083.
[87] P. Le, et al., Non-coding RNAs in cancer diagnosis and therapy: focus on lung cancer, Cancers (Basel) 13 (6) (2021).
[88] L.S. Kristensen, et al., The emerging roles of circRNAs in cancer and oncology, Nat. Rev. Clin. Oncol. 19 (3) (2022) 188–206.
[89] J. He, et al., Circular RNAs and cancer, Cancer Lett. 396 (2017) 138–144.
[90] S. Beyes, N.G. Bediaga, A. Zippo, An epigenetic perspective on intra-tumour heterogeneity: novel insights and new challenges from multiple fields, Cancers (Basel) 13 (19) (2021).
[91] T.K. Kelly, D.D. De Carvalho, P.A. Jones, Epigenetic modifications as therapeutic targets, Nat. Biotechnol. 28 (10) (2010) 1069–1078.
[92] P. Bojang Jr., K.S. Ramos, The promise and failures of epigenetic therapies for cancer treatment, Cancer Treat. Rev. 40 (1) (2014) 153–169.
[93] M.A. Dawson, The cancer epigenome: concepts, challenges, and therapeutic opportunities, Science 355 (6330) (2017) 1147–1152.
[94] T. Asano, Drug resistance in cancer therapy and the role of epigenetics, J. Nippon Med. Sch. 87 (5) (2020) 244–251.
[95] K. Kaminska, et al., Prognostic and predictive epigenetic biomarkers in oncology, Mol .Diagn. Ther. 23 (1) (2019) 83–95.
[96] A. Nishiyama, M. Nakanishi, Navigating the DNA methylation landscape of cancer, Trends Genet. 37 (11) (2021) 1012–1027.
[97] S. Romero-Garcia, H. Prado-Garcia, A. Carlos-Reyes, Role of DNA methylation in the resistance to therapy in solid tumors, Front. Oncol. 10 (2020) 1152.
[98] J. Ahmed II, et al., P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease, Heliyon 8 (6) (2022) e09777.
[99] M. Duruisseaux, M. Esteller, Lung cancer epigenetics: From knowledge to applications, Semin. Cancer Biol. 51 (2018) 116–128.
[100] G. Deng, et al., Regional hypermethylation and global hypomethylation are associated with altered chromatin conformation and histone acetylation in colorectal cancer, Int. J. Cancer 118 (12) (2006) 2999–3005.
[101] G.C. Hon, et al., Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer, Genome Res. 22 (2) (2012) 246–258.
[102] A.M. Hosey, et al., Molecular basis for estrogen receptor alpha deficiency in BRCA1-linked breast cancer, J. Natl. Cancer Inst. 99 (22) (2007) 1683–1694.
[103] J. Wen, et al., Decreased BRCA1 confers tamoxifen resistance in breast cancer cells by altering estrogen receptor-coregulator interactions, Oncogene 28 (4) (2009) 575–586.
[104] H. Jo, et al., HDAC2 as a target for developing anti-cancer drugs, Comput. Struct. Biotechnol. J. 21 (2023) 2048–2057.
[105] H. Yang, et al., Overexpression of histone deacetylases in cancer cells is controlled by interplay of transcription factors and epigenetic modulators, FASEB J. 28 (10) (2014) 4265–4279.
[106] Z. Yao, et al., Chromatin-modifying drugs and metabolites in cell fate control, Cell Prolif. 53 (11) (2020) e12898.
[107] L.S. Zeng, et al., Overexpressed HDAC4 is associated with poor survival and promotes tumor progression in esophageal carcinoma, Aging (Albany NY) 8 (6) (2016) 1236–1249.
[108] Y. Yao, W. Dai, Genomic instability and cancer, J Carcinog. Mutagen. 5 (2014).
[109] A. Hirukawa, et al., Reduction of global H3K27me(3) enhances HER2/ErbB2 targeted therapy, Cell Rep. 29 (2) (2019) 249–257, e8.
[110] X. Zhou, et al., Non-coding RNA in cancer drug resistance: Underlying mechanisms and clinical applications, Front. Oncol. 12 (2022), 951864.
[111] J. An, W. Lv, Y. Zhang, LncRNA NEAT1 contributes to paclitaxel resistance of ovarian cancer cells by regulating ZEB1 expression via miR-194, Onco. Targets Ther. 10 (2017) 5377–5390.
[112] G. Anandappa, et al., miR-31-3p expression and benefit from Anti-EGFR inhibitors in metastatic colorectal cancer patients enrolled in the prospective Phase II PROSPECT-C trial, Clin. Cancer Res. 25 (13) (2019) 3830–3838.
[113] R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nat. Rev. Drug Discov. 16 (3) (2017) 203–222.
[114] A. Bhan, M. Soleimani, S.S. Mandal, Long noncoding RNA and cancer: a new paradigm, Cancer Res. 77 (15) (2017) 3965–3981.
[115] P. Martini, et al., lncRNAs as novel indicators of Patients' prognosis in stage I epithelial ovarian cancer: a retrospective and multicentric study, Clin. Cancer Res. 23 (9) (2017) 2356–2366.
[116] H. Ling, M. Fabbri, G.A. Calin, MicroRNAs and other non-coding RNAs as targets for anticancer drug development, Nat. Rev. Drug Discov. 12 (11) (2013) 847–865.
[117] X. Deng, et al., Long noncoding RNA CCAL transferred from fibroblasts by exosomes promotes chemoresistance of colorectal cancer cells, Int. J. Cancer 146 (6) (2020) 1700–1716.
[118] J.T. Miao, et al., LncRNA ANRIL affects the sensitivity of ovarian cancer to cisplatin via regulation of let-7a/HMGA2 axis, Biosci. Rep. 39 (7) (2019).
[119] M. Luo, et al., Drug resistance in colorectal cancer: An epigenetic overview, Biochim. Biophys. Acta Rev. Cancer 1876 (2) (2021), 188623.
[120] L.X. Wu, et al., Interindividual epigenetic variation in ABCB1 promoter and its relationship with ABCB1 expression and function in healthy Chinese subjects, Br. J. Clin. Pharmacol. 80 (5) (2015) 1109–1121.
[121] T.L. Clarke, et al., Histone lysine methylation dynamics control EGFR DNA Copy-number amplification, Cancer Discov. 10 (2) (2020) 306–325.
[122] J.K. Christman, 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy, Oncogene 21 (35) (2002) 5483–5495.
[123] D.S. Hong, et al., Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours, Br. J. Cancer 122 (11) (2020) 1630–1637.
[124] Y. Li, M.D. Disney, Precise small molecule degradation of a noncoding RNA identifies cellular binding sites and modulates an oncogenic phenotype, ACS Chem. Biol. 13 (11) (2018) 3065–3071.
[125] S.P. Velagapudi, et al., Design of a small molecule against an oncogenic noncoding RNA, Proc. Natl. Acad. Sci. U.S.A., 113 (21) (2016) 5898–5903.
[126] D. Subramaniam, et al., DNA methyltransferases: a novel target for prevention and therapy, Front. Oncol. 4 (2014) 80.
[127] C. Hu, et al., DNA methyltransferase inhibitors combination therapy for the treatment of solid tumor: mechanism and clinical application, Clin. Epigenetic. 13 (1) (2021) 166.
[128] A. Gnyszka, Z. Jastrzebski, S. Flis, DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer, Anticancer Res. 33 (8) (2013) 2989–2996.
[129] G. Arun, S.D. Diermeier, D.L. Spector, Therapeutic targeting of long non-coding RNAs in cancer, Trends Mol. Med. 24 (3) (2018) 257–277.
[130] X. Zhang, et al., Role of non-coding RNAs and RNA modifiers in cancer therapy resistance, Mol. Cancer 19 (1) (2020) 47.
[131] Y. Cheng, et al., Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials, Signal Transduct Target Ther. 4 (2019) 62.
[132] M. Zhou, et al., Combining histone deacetylase inhibitors (HDACis) with other therapies for cancer therapy, Eur. J. Med. Chem. 226 (2021), 113825.
[133] M. Huang, et al., Histone acetyltransferase inhibitors: An overview in synthesis, structure-activity relationship and molecular mechanism, Eur. J. Med. Chem. 178 (2019) 259–286.
[134] J. Yu, et al., DNA methyltransferase expression in triple-negative breast cancer predicts sensitivity to decitabine, J. Clin. Invest. 128 (6) (2018) 2376–2388.
[135] L.A. Cowan, S. Talwar, A.S. Yang, Will DNA methylation inhibitors work in solid tumors? A review of the clinical experience with azacitidine and decitabine in solid tumors, Epigenomics 2 (1) (2010) 71–86.
[136] J.F. Linnekamp, et al., Clinical and biological effects of demethylating agents on solid tumours - a systematic review, Cancer Treat. Rev. 54 (2017) 10–23.
[137] S. Fu, et al., Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer, Cancer 117 (8) (2011) 1661–1669.
[138] F. Fang, et al., A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer, Cancer 116 (17) (2010) 4043–4053.
[139] D. Matei, et al., Epigenetic resensitization to platinum in ovarian cancer, Cancer Res. 72 (9) (2012) 2197–2205.
[140] R.A. Juergens, et al., Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer, Cancer Discov. 1 (7) (2011) 598–607.
[141] R.M. Connolly, et al., Combination epigenetic therapy in advanced breast cancer with 5-Azacitidine and Entinostat: A Phase II National Cancer Institute/Stand up to cancer study, Clin. Cancer Res. 23 (11) (2017) 2691–2701.
[142] C.D. DiNardo, et al., Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia, Blood 133 (1) (2019) 7–17.
[143] M. Duvic, et al., Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL), Blood 109 (1) (2007) 31–39.
[144] E.A. Olsen, et al., Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma, J. Clin. Oncol. 25 (21) (2007) 3109–3115.
[145] O. Khan, N.B. La Thangue, Drug insight: histone deacetylase inhibitor-based therapies for cutaneous T-cell lymphomas, Nat. Clin. Pract. Oncol. 5 (12) (2008) 714–726.
[146] G.R. Blumenschein Jr., et al., Phase II trial of the histone deacetylase inhibitor vorinostat (Zolinza, suberoylanilide hydroxamic acid, SAHA) in patients with recurrent and/or metastatic head and neck cancer, Invest. New Drugs 26 (1) (2008) 81–87.
[147] D. Bradley, et al., Vorinostat in advanced prostate cancer patients progressing on prior chemotherapy (National Cancer Institute Trial 6862): trial results and interleukin-6 analysis: a study by the Department of Defense Prostate Cancer Clinical Trial Consortium and University of Chicago Phase 2 Consortium, Cancer 115 (23) (2009) 5541–5549.
[148] E. Galanis, et al., Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study, J. Clin. Oncol. 27 (12) (2009) 2052–2058.
[149] S.C. Modesitt, et al., A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study, Gynecol. Oncol. 109 (2) (2008) 182–186.

[150] A.M. Traynor, et al., Vorinostat (NSC# 701852) in patients with relapsed non-small cell lung cancer: a Wisconsin Oncology Network phase II study, J. Thorac. Oncol. 4 (4) (2009) 522–526.
[151] S.J. Whittaker, et al., Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma, J. Clin. Oncol. 28 (29) (2010) 4485–4491.
[152] B. Coiffier, et al., Romidepsin for the treatment of relapsed/refractory peripheral T-cell lymphoma: pivotal study update demonstrates durable responses, J. Hematol. Oncol. 7 (2014) 11.
[153] F.M. Iwamoto, et al., A phase I/II trial of the histone deacetylase inhibitor romidepsin for adults with recurrent malignant glioma: North American brain tumor consortium study 03-03, Neuro. Oncol. 13 (5) (2011) 509–516.
[154] L.R. Molife, et al., Phase II, two-stage, single-arm trial of the histone deacetylase inhibitor (HDACi) romidepsin in metastatic castration-resistant prostate cancer (CRPC), Ann. Oncol. 21 (1) (2010) 109–113.
[155] G.A. Otterson, et al., Phase II study of the histone deacetylase inhibitor Romidepsin in relapsed small cell lung cancer (Cancer and Leukemia Group B 30304), J. Thorac. Oncol. 5 (10) (2010) 1644–1648.
[156] R.L. Piekarz, et al., Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma, Blood 117 (22) (2011) 5827–5834.
[157] W.M. Stadler, et al., A phase II study of depsipeptide in refractory metastatic renal cell cancer, Clin. Genitourin. Cancer 5 (1) (2006) 57–60.
[158] R.P. Whitehead, et al., Phase II trial of romidepsin (NSC-630176) in previously treated colorectal cancer patients with advanced disease: a Southwest Oncology Group study (S0336), Invest. New Drugs 27 (5) (2009) 469–475.
[159] Tachyon Therapeutics, I. *A Study to assess the safety, pharmacokinetics, and antitumor activity of oral TACH101 in participants with advanced or metastatic cancer*. 2023 11 October 2023]; Available from: https://clinicaltrials.gov/study/NCT05076552.
[160] C. Chandhasin, et al., TACH101, a first-in-class pan-inhibitor of KDM4 histone demethylase, Anticancer Drugs (2023).
[161] A. Khvorova, J.K. Watts, The chemical evolution of oligonucleotide therapies of clinical utility, Nat. Biotechnol. 35 (3) (2017) 238–248.
[162] S. Ochoa, V.T. Milam, Modified nucleic acids: expanding the capabilities of functional oligonucleotides, Molecules 25 (20) (2020).
[163] M.A. Cortez, et al., Role of miRNAs in immune responses and immunotherapy in cancer, Genes Chromosomes Cancer 58 (4) (2019) 244–253.
[164] M.A. Smolle, et al., Noncoding RNAs and immune checkpoints-clinical implications as cancer therapeutics, FEBS J. 284 (13) (2017) 1952–1966.
[165] A.W. Whitehurst, et al., Synthetic lethal screen identification of chemosensitizer loci in cancer cells, Nature 446 (7137) (2007) 815–819.
[166] S.L. Topalian, C.G. Drake, D.M. Pardoll, Immune checkpoint blockade: a common denominator approach to cancer therapy, Cancer Cell 27 (4) (2015) 450–461.
[167] A.F. Setiadi, et al., Epigenetic enhancement of antigen processing and presentation promotes immune recognition of tumors, Cancer Res. 68 (23) (2008) 9601–9607.
[168] A.C. West, R.W. Johnstone, New and emerging HDAC inhibitors for cancer treatment, J. Clin. Invest. 124 (1) (2014) 30–39.
[169] P. Sharma, J.P. Allison, Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential, Cell 161 (2) (2015) 205–214.
[170] M.J. Topper, et al., The emerging role of epigenetic therapeutics in immuno-oncology, Nat. Rev. Clin. Oncol. 17 (2) (2020) 75–90.
[171] M. Agathocleous, et al., Ascorbate regulates haematopoietic stem cell function and leukaemogenesis, Nature 549 (7673) (2017) 476–481.
[172] K. Blaschke, et al., Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells, Nature 500 (7461) (2013) 222–226.
[173] L. Kong, et al., The DNA methylation-regulated MCTP1 activates the drug-resistance of esophageal cancer cells, Aging (Albany NY) 13 (3) (2021) 3342–3352.
[174] J.C. Hahne, N. Valeri, Non-Coding RNAs and resistance to anticancer drugs in gastrointestinal tumors, Front. Oncol. 8 (2018) 226.
[175] B.B. Liau, et al., Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance, Cell Stem Cell 20 (2) (2017) 233–246, e7.
[176] H. Dombret, et al., International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts, Blood 126 (3) (2015) 291–299.
[177] L. Pleyer, et al., Azacitidine front-line in 339 patients with myelodysplastic syndromes and acute myeloid leukaemia: comparison of French-American-British and World Health Organization classifications, J. Hematol. Oncol. 9 (2016) 39.
[178] M. Lubbert, et al., A multicenter phase II trial of decitabine as first-line treatment for older patients with acute myeloid leukemia judged unfit for induction chemotherapy, Haematologica 97 (3) (2012) 393–401.
[179] V. Santini, et al., The clinical value of decitabine monotherapy in patients with acute myeloid leukemia, Adv. Ther. 39 (4) (2022) 1474–1488.
[180] M. Duvic, J. Vu, Vorinostat in cutaneous T-cell lymphoma, Drugs Today (Barc.) 43 (9) (2007) 585–599.
[181] B. Coiffier, et al., Results from a pivotal, open-label, phase II study of romidepsin in relapsed or refractory peripheral T-cell lymphoma after prior systemic therapy, J. Clin. Oncol. 30 (6) (2012) 631–636.