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Comprehensive Insights into the Epigenetic Landscape of Cancer: Mechanisms, Therapeutic Targets, and Clinical Implication

BY

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Abstract

DNA methylation, histone modification, and chromatin remodeling are examples of epigenetic modifications that play a critical role in the development of cancer. These modifications silence tumor suppressor genes, activate oncogenes, and interfere with the control of the cell cycle and apoptosis. Long and micro non-coding RNAs, two types of non-coding RNAs that are important in epigenetic regulation, have the potential to be used as biomarkers for cancer prognosis and diagnosis. In order to address therapeutic resistance and enhance treatment response, epigenetic medicines like as histone deacetylase inhibitors and DNA methyl transferase are being investigated in the clinical setting, especially in conjunction with conventional treatments. Future studies seek to improve patient outcomes by advancing our understanding of cancer biology by integrating epigenetic data with genomic and transcriptomic data.

Keywords: *Epigenetic alterations, Cancer development, DNA methylation, Histone modification, Chromatin remodeling*

1. Introduction

In the early 1940s, Conrad Waddington defined "epigenetics" as "the branch of biology that studies the direct interactions between genes and their products, which bring the phenotype into being" [1]. The word "epigenetics" back then covered all biological mechanisms that regulate how a genotype manifests itself to produce a certain phenotype. But because genetics advanced so swiftly, the term's meaning was constrained. Epigenetics is now commonly defined as "the study of heritable changes in gene function that occur without changes in the DNA sequence, whether through mitosis or meiosis" [2]. Most recent research associates epigenetic modifications with covalent modifications of DNA bases, post-translational modifications of histone amino-terminal tails, and variances in histones. It is necessary to thoroughly analyze the current understanding of epigenetics since these changes in epigenetics are also necessary for the expression and silencing of noncoding sequences [3].

2. Overview of epigenetic alterations in cancer

Genetic and epigenetic alterations in DNA are the main source of the changes in gene expression that cause aging and cancer. These modifications, which include nucleosome remodeling, microRNAs, DNA methylation, and histone modifications, commonly manifest as epigenetic abnormalities in human cancers [4]. These epigenetic regulators play a critical role in controlling gene expression and preserving cellular homeostasis. A complex interplay between genetic and epigenetic mechanisms underlies abnormal metabolic and biochemical processes in cancer. Widespread alterations in gene expression can result from mutations in the genes that regulate epigenetic processes, such

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as DNA methylation and histone modification, which may play a role in the development of tumors. Tumor suppressor genes may go silent as a result of these alterations, or they may cause oncogenes to become active, hastening the spread of cancer. Targeting these epigenetic modifications in therapeutic approaches has shown promise, especially in hematological malignancies including lymphomas and preleukemia [5]. The capacity to reverse aberrant epigenetic patterns represents one potential therapeutic avenue for malignancies resulting from these changes. Emerging data suggests that epigenetic therapy may be used to treat solid tumors, while further research in this field is necessary. Hypoxia is a common feature of the tumor microenvironment, and it can lead to specific epigenetic changes that hasten the growth of cancer. Hypoxia-induced alterations in DNA methylation, histone modifications, and microRNA expression may promote a more aggressive tumor phenotype, which might aid in metastasis and treatment.

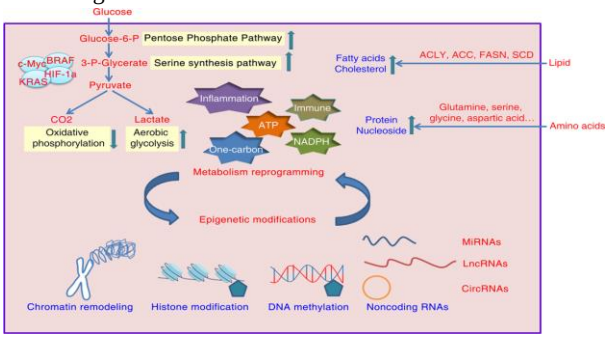

Fig 1 .Metabolic reprogramming pathways and epigenetic modification marks interplay in cancer [7]

3. DNA Methylation in Cancer

Gene expression depends critically on DNA methylation, and therapy aimed at slowing the course of cancer is supported by changes in DNA methylation [8]. This knowledge suggests that it could be a cancer biomarker and contribute to the creation of effective treatment strategies. One of the essential cellular transcription regulation processes that also contribute to the development of cancer is DNA methylation [9]. Through hypo-or hypermethylation, abnormal DNA methylation causes cells to acquire carcinogenic characteristics. This modification is a component of epigenetic changes. Tumor suppressor gene silence is linked to hypermethylation, and this change has revealed the universal cause of oncogenes [10]. DNA methylation mostly impacts silenced genes and activates oncogenes in the context of cancer. DNA methylation is mediated by DNMTs, which move a methyl group from the universal methyl donor S-adenosyl methionine (SAM) to the cytosine ring. 5-methylcytosine is created when methylation takes place at cytosine's fifth carbon. This alteration has the potential to cause transcriptional repression and significantly affect gene expression by altering the binding affinity of transcription factors or drawing in proteins that recognize methylation DNA [11].

Humans possess four types of DNMTs, each with distinct functions:

1. **DNMT1**: DNMT1, also known as the maintenance methyltransferase, is mostly in charge of transferring methylation patterns from the parent DNA strands to the daughter DNA strands while DNA replicates. This preserves the genome's epigenetic landscape by ensuring that the methylation patterns are consistently passed down through cell divisions [12]. Because of its strong affinity for hemi methylated DNA, DNMT1 is a perfect fit for its function in maintenance methylation.
2. **DNMT2**: The major function of DNMT1, also known as the maintenance methyltransferase, is to transfer methylation patterns from the parent DNA strands to the daughter DNA strands during DNA replication. Ensuring that methylation patterns are consistently passed down through cell divisions safeguards the genome's epigenetic landscape [12]. Because of its strong affinity for hemi-methylated DNA, DNMT1 is perfectly suited for its function in maintenance methylation.
3. **DNMT3a** and **DNMT3b**: Known as de novo methyltransferases, these enzymes are responsible for the creation of new DNA methylation patterns throughout the early phases of development. DNMT3a and DNMT3b are critical for the establishment of tissue-specific methylation patterns during embryogenesis because they have the ability to methylate unmethylated DNA, whereas DNMT1 primarily functions on hemi-methylated DNA. DNMT3a and DNMT3b are essential for normal development, and mutations in these enzymes have been connected to a variety of cancers and developmental issues.

Table1. Function of DNMTs in DNA methylation and their role in cancer

DNMTs	Function in DNA methylation	Involve in cancer
DNMT1	During DNA replication it maintains methyl group in the semi-methylated stand of DNA	Esophageal cancer, pancreas cancer, Colon cancer
DNMT2	It plays role in cytosine methylation	Breast cancer
DNMT3a	It conveyances methyl group in specific CpG side of DNA	Acute myeloid leukaemia, liver cancer, lymphoma
DNMT3b	It is involved in de novo DNA methylation and works with RNA Pol-II and H3K36me3	Acute myeloid leukemia, breast cancer, prostate cancer

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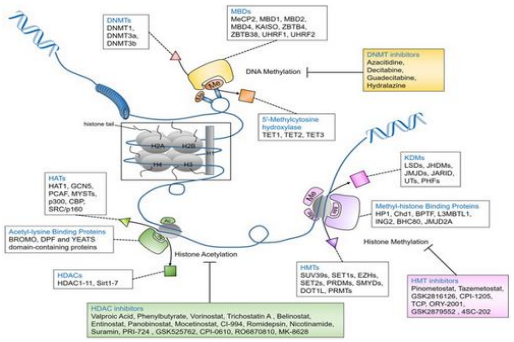


Fig 2.Significance of Histone Methylation and DNA Methylation in Epigenetics Reproduced with permission under Creative Commons CC BY 4.0 license from 9 (Copyright 2019 The Authors)

Mechanisms of DNA methylation

Two more common epigenetic processes that limit gene expression are histone modification and non-coding RNA-mediated control of gene expression. One of these mechanisms, DNA methylation, inhibits gene expression in a number of different ways. Most of the time, DNA is methylated at the cytosine C5 position in the CpG dinucleotide (CpG sites). The methylation-prone CpG sites are not dispersed equally throughout the genome; they have the potential to unite to create CpG islands. DNA sequences containing more than 55% G and C nucleotides, at least 500 base pairs in length, and a ratio of more than 65% between the actual and predicted numbers of CpG sites evenly dispersed throughout the genome are known as CpG islands [14].

The most functionally significant regions are CpG islands and their neighboring areas within 2 kb, as their methylation or demethylation significantly alters the expression level of nearby genes [15]. Additionally, there are isolated regions (shelves) that are separated from the surrounding areas and the remainder of the genome (sea) by a distance of only 2 kb, where CpG sites are uncommon and dispersed very evenly. CpG islands are found in around 70% of gene promoters, and their involvement in regulating gene expression is determined by these islands.

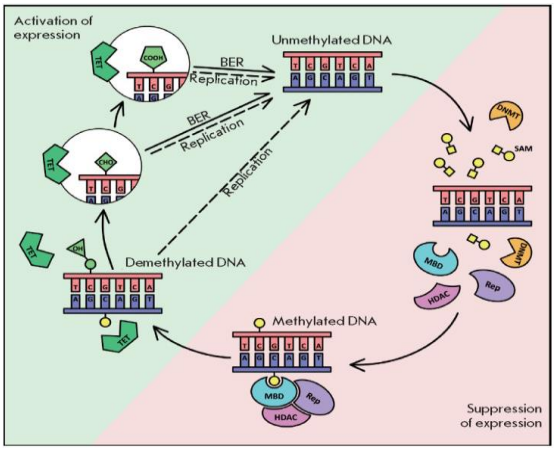


Fig 3.DNA Methylation as an Epigenetic Mechanism for Regulating Gene Expression [16]

3.2. **Role of DNA methylation in gene silencing**

Both normal and malignant cells have various levels of regulation over DNA methylation. A family of enzymes known as DNA methyltransferases (DNMTs) adds methyl groups throughout this process. DNA methylation and transcriptional activity are also influenced by the chromatin structure surrounding gene promoters. The regulation of chromatin accessibility and transcription factor binding, which in turn impacts gene expression, is greatly influenced by parameters such as nucleosome location and histone acetylation [16].

DNA methyltransferase

Enzymes called DNMTs make it quicker for methyl groups to be added to DNA's cytosine residues. The three main DNMTs found in mammalian cells are DNMT1, DNMT3a, and DNMT3b [17]. While DNMT3a and DNMT3b are involved in the establishment of new, or de novo, methylation patterns, DNMT1 is primarily in charge of preserving preexisting methylation patterns during mouse development. Interestingly, DNMT1 may collaborate with DNMT3b to maintain aberrant gene hypermethylation in cancer cells, suggesting that its function in these cells goes beyond simple methylation pattern maintenance. Therapeutic approaches that target DNA methylation in cancer may benefit from this cooperative interaction [18].

Chromatin structure

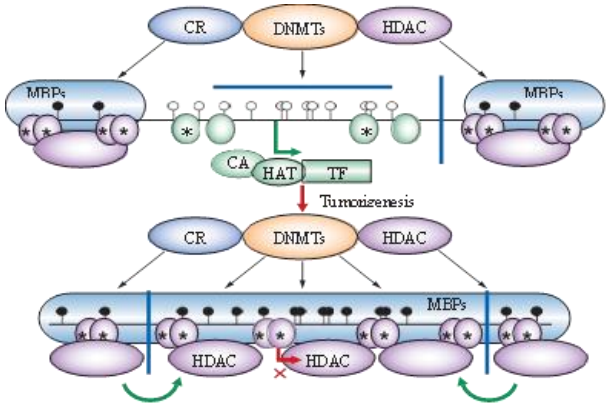
Even while DNA methylation is essential for regulating gene activity, transcription cannot be repressed by DNA methylation on its own. The transcription or silencing of genes is also strongly influenced by the local chromatin structure [18]. By attracting histone deacetylases (HDACs) and other chromatin-binding proteins to promoter areas, where they maintain histone deacetylation, DNMTs contribute in the silence of genes. Unmethylated, transcriptionally active genes have a more open chromatin configuration, whereas methylated, quiet genes are linked to strongly compacted chromatin that hinders transcription [19]. Histone acetylation is essential for controlling chromatin shape and gene transcription. Nucleosomes have an impact on transcription and chromatin structure because they are made of DNA encircled by histone proteins.

While hypermethylated CpG islands cause tighter nucleosome compaction that blocks transcriptional machinery, unmethylated CpG islands allow nucleosomes to be more accessible to transcriptional factors. Recent studies have demonstrated that methylation of some histone H3 residues, including lysine 9, is connected to transcriptional repression, whereas methylation of lysine 4 is connected to active transcription. Transcription factors and regulatory coactivators can bind to chromatin when histones are acetylated, keeping it open. On the other hand, HDACs preserve transcriptional silence by retaining histones deacetylated and blocking access to the transcriptional machinery, assisted by DNMTs and methyl cytosine-binding proteins [20].

Table 2.Selected genes hyper methylated in sporadic cancers

Gene or gene product	Function	Tumor type
Rb	Cell-cycle regulation	Retinoblastoma
APC	Wnt signal transduction	Colorectal and other cancers
p14/ARF	Cell-cycle regulation	Colorectal cancer
p15/CDKN2B	Cell-cycle regulation	Leukemia
p16/CDKN2A	Cell-cycle regulation	Various cancers
BRCA1	DNA repair	Breast, ovarian cancer
VHL	Tumor suppressor[a]	Renal cell cancers
hMLH1	DNA mismatch repair	Colorectal, gastric, endometrial cancers
ER-α	Estrogen receptor-α	Breast, colorectal, other cancers

Fig 4.Impact of DNA Methylation and Chromatin Structure on Gene Transcription in Normal and Tumor Cells [20]



3.2.3. Cancer-specific DNA methylation changes

Three to five percent of cytosines are methylated in the majority of mammalian tissues. Global DNA hypo-methylation causes a small to moderate reduction in the amount of methylated cytosines in tumor genomes, with an average loss of 5% to 20% of 5-methylcytosine bases. It has proven difficult to identify certain genes or biochemical pathways that are directly affected by the decrease in methylation because of how widespread this loss is [21].

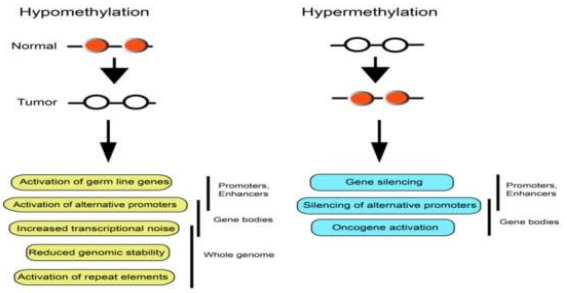


Fig 5.Genome-scale consequences of DNA hypo methylation and DNA hyper methylation in cancer [21]

3.3. Tumor-Driving DNA Hyper methylation Events

In addition to the majority of primary and metastatic cancers, premalignant lesions like actinic keratosis in the skin or aberrant crypt foci in the colon are also shown to have genome-wide hyper methylation of CpG islands. When hypermethylation affects regulatory gene sequences, including enhancer or promoter regions, where it usually results in gene silencing, its involvement in driving tumor genesis becomes most apparent. On the other hand, hypermethylation in CpG-rich areas of gene bodies can lead to one of two possible results. It can suppress one of a gene's many alternative promoters, changing the way a particular transcript is expressed, or it can be linked, more broadly, to higher levels of gene expression. When hypermethylation takes place in oncogenes, it may activate these genes, which in turn may promote carcinogenesis. On the other hand, hypermethylation of CpG islands in promoter regions is more frequently responsible for gene silence. If methylation-induced gene silence impacts genes linked to the "hallmarks of cancer," such as angiogenesis, cell adhesion, invasion and metastasis, DNA repair and genomic integrity, and anti-inflammatory responses, it is especially important. [22]

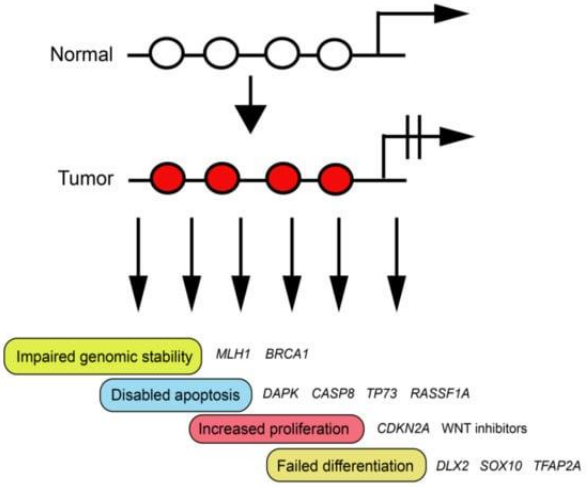


Fig 6.Potential Tumor-Driving Effects of CpG Island Hyper methylation [23]

3.1 Clinical applications of DNA methylation markers

A methyl group is added to the fifth carbon of the cytosine residues in the CpG dinucleotide to cause DNA methylation, a significant epigenetic alteration. This mechanism is essential for controlling gene expression and preserving the integrity of the genome. A number of malignancies and other disorders

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are characterized by aberrant DNA methylation patterns, which include hypo- and hypermethylation of oncogenes and tumor suppressor genes, respectively. As a result, DNA methylation markers have become attractive instruments for use in clinical settings, such as prognostication, therapeutic targeting, and disease detection.

3.1.1 Diagnostic Applications

The diagnosis of cancer benefits greatly from the use of DNA methylation indicators. Tumor suppressor genes' promoter regions may become hyper methylated, which may silence the gene and assist in the development of tumors. For example, it has been noted that certain cancer types, such as lung and colorectal cancer, have methylation of the promoter of the CDKN2A gene, which encodes the tumor suppressor protein p16 [24]. Such hyper methylation events can be found in body fluids like blood or urine, which provides a non-invasive way to find cancer early [25] .The diagnosis of colorectal cancer via the methylation analysis of the SEPT9 gene in plasma, is one of the most important clinical uses of DNA methylation indicators. The FDA has approved this test, called the Epi proColon® test, which demonstrates DNA methylation's potential as a biomarker for non-invasive cancer screening [26].

3.1.2 Prognostic Applications

DNA methylation indicators may provide useful prognostic information in addition to diagnosis. Patient outcomes in a variety of malignancies have been linked to the methylation status of specific genes. For example, in patients with glioblastoma, hypermethylation of the MGMT (O6-methylguanine-DNA methyltransferase) gene promoter is linked to improved responses to alkylating drugs because it inhibits the repair of alkylated DNA lesions, increasing the efficacy of chemotherapy [27]. Likewise, a poor prognosis in cases of sporadic breast cancer has been associated with the methylation of the BRCA1 gene, which is well-known for its involvement in hereditary breast and ovarian cancers. BRCA1 promoter methylation is a crucial prognostic marker since it is associated with aggressive tumor behavior and poor survival outcomes [28].

3.1.3 Therapeutic Targeting

DNA methylation is a desirable target for therapeutic intervention due to its reversible nature. Through demethylating the promoter regions of silenced tumor suppressor genes, medications referred to as DNA methyltransferase inhibitors (DNMT inhibitors) may reactivate such genes. Targeting DNA methylation in cancer therapy has potential, as evidenced by the approval of drugs like 5-azacytidine and decitabine for the treatment of certain leukemias and myelodysplastic syndromes [29].

4. Histone Modification in Cancer

4.1 Overview of histone modifications (e.g. acetylation, methylation, phosphorylation)

Proteins known as "writers" and "erasers" are responsible for catalyzing histone modifications, which are covalent post-translational changes to histone tails, including H2A, H2B, H3, and H4. At the moment, a number of well-studied histone modifications—including those linked to active transcription, such as H3K4me3 and H3K36me3, and repressed genes, such as H3K27me3, H3K9me2/3, and H4K20me3—are implicated in the development of cancer [30]. The most common changes found in histone tails are phosphorylation, acetylation, and histone methylation. Numerous additional modifications, including ubiquitination, acetylation, propionylation, crotonylation, and formylation, have also been identified [31].

4.1.1 Histone methylation

In histone methylation, methyl groups are transferred from S-adenosyl methionine (SAM) to the arginine (R) and lysine (K) residues of the H3 or H4 tails, respectively, using lysine methyltransferases (KMTs) or arginine methyltransferases (PRMTs). Histone lysine residues' methyl groups are eliminated by lysine demethylases (KDMs) [32]. Histone-binding proteins are primarily recruited or their recruitment is inhibited when histone methylation takes place. For instance, H3K4me3 attracts activating proteins to gene promoters, such as transcription factors (TFs), while H3K4 inhibits the recruitment of repressors, such as the nucleosome remodeling and deacetylases (NuRD) complex [33]. Gene suppression is the result of H3K9me2/3's particular binding to chromo domain proteins, such as the heterochromatin protein 1 (HP1) family, which forms the higher-order architecture of heterochromatin [34]. Cancer cells typically have mutations in their histone genes and histone modifier enzymes, which alter the chromatin methylation patterns and cause tumor growth and metastasis. Mutations in several enzymes involved in histone methylation have been found, according to analysis of The Cancer Genome Atlas (TCGA) databases [35]. One of the well-known disordered histone modifications that causes aberrant gene expression and genome stability in cancer is loss or gain of function of H3K27me3. This is typically brought on by mutations in the gene encoding the histone methyltransferase enhancer of zeste homologue 2 (EZH2). Furthermore, mutant histones with characteristics that promote tumor growth, such as H3K27M/I, can also affect the enzymatic activity of EZH2 [36]. Even with the progress made, a deeper comprehension of the aberrant histone methylation patterns in cancers is still required to clarify the molecular processes underlying tumorigenicity and create innovative targeted therapies or combination treatments.

4.1.2 Histone acetylation

Histone acetylation and deacetylation are processes that involve adding or removing acetyl groups from the lysine residues of tails that protrude from the histone core of the nucleosome. These processes are linked to a number of important cellular processes, including RNA transcription, DNA replication, and DNA damage repair [37]. Enzymes having histone acetyl transferase (HAT) or histone deacetylase (HDAC) activity usually catalyze these processes [38]. One of the fastest post-translational modifications (PTMs) in terms of dynamics is histone acetylation, which is quicker than methylation but slower than phosphorylation [39]. Changes in histone acetylation have been linked to the development of cancer, according to numerous studies. Through their alteration of histone acetylation and regulation

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of the expression of oncogenes such p300 and CBP, HDAC overexpression and increased activity have been identified as drivers of tumor formation and metastasis [40]. But it has also been demonstrated that p300 and CBP function as tumor suppressors in a number of solid tumors as well as hematological malignancies [41]. These results imply that more research is needed to understand the functions of p300 and CBP, two other HATs, in cancer. Furthermore, HDAC1, a part of the NuRD complex, has the ability to catalyze the deacetylation of H3K27 at the promoter of the STAT1 gene, resulting in an immunosuppressive milieu that facilitates the growth of glioma stem-like cells (GSCs) [42]. The US FDA has currently approved four HDAC inhibitors for the treatment of cancer: panobinostat for multiple myeloma, istodax and beleodap for T cell lymphoma, and vorinostat for that condition [43]. Given that histone acetylation is reversible, new techniques or combination strategies must be created in order to return the histone acetylation status of cancer cells to normal.

4.1.3 Histone phosphorylation

Phosphorylation of histones another modification of histones, phosphorylation, alters the structure of the chromatin by introducing a negative charge mostly to the tyrosine (Y), serine (S), and threonine (T) of histone tails. This enables the interaction with transcription factors to control the expression of genes linked to cell cycle and proliferation [44]. Aberrant histone phosphorylation has the same potential to influence tumor development and metastasis as histone methylation and acetylation. One well-known alteration that is assumed to be a cancer biomarker is H3S10P, which is regulated by several kinases and is connected with positive regulation of transcription. By increasing H4S1 phosphorylation and suppressing slug expression, deletion of N-α-acetyltransferase D (NatD) suppresses the epithelial mesenchyme transition (EMT) in lung cancer [45]. In mouse embryonic stem cells (mESCs), phosphorylation of H3.3 at serine 31 can increase p300 activity and histone acetylation [46]. This provides new insights into the development of cancer drugs by indicating that histone phosphorylation is involved in several crosstalk processes with other histone changes.

4.2 Cancer-specific histone modification changes

Proteins known as "writers" and "erasers" are responsible for catalyzing histone modifications, which are covalent post-translational changes to histone tails, including H2A, H2B, H3, and H4. At the moment, a number of well-studied histone modifications—including those linked to active transcription, such as H3K4me3 and H3K36me3, and repressed genes, such as H3K27me3, H3K9me2/3, and H4K20me3—are implicated in the development of cancer [47]. Although an extensive range of histone modifications has been described, little is known about their functional significance. The most common changes seen in histone tails are methylation, acetylation, and phosphorylation; numerous additional modifications, including ubiquitination, lactylation, propionylation, crotonylation, and formylation, have also been identified [48]. On the tails of the four core histones, H2A, H2B, H3, and H4, several changes may take place. Additionally, additional changes can also be applied to variations of H2A and H3, including methylation or phosphorylation on CENP-A, phosphorylation or biotinylation on H3.3, and acetylation or phosphorylation on H2A.X. The most frequent modifications to histones are methylation and acetylation, which often happen on the same lysine sites of the four histones (H2AK5/13,H2BK5/46/108,H3K4/9/14/23/27/36/56/64/79/122, and H4K5/8/12/20/31/79). Furthermore, it has been observed that a number of amino acid sites on histone tails, such as H2AK13me/ac/ar/bio, H3K9me/ac/cr, H3K14me/ac/pr/bu, H3K18me/ac/la/cr, H4K5me/ac/pr/bu/la, H4K8me/ac/pr/bio/cr, and H4K12me/ac/pr/bu/bio, are frequently modified with more than two distinct modifications. These sites are involved in the regulation of genes and the determination of cell fate.

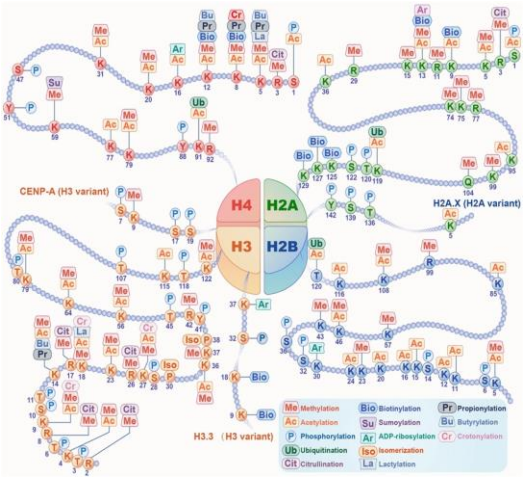

Fig 7.Histone modifications [48]

4.3 Clinical applications of histone modification markers

4.3.1 Cancer Diagnosis:

- **Histone Methylation Patterns:** Early cancer identification can be aided by the use of aberrant methylation patterns, such as decreased H3K27me3 or elevated H3K4me3, to differentiate between malignant and normal tissues [49]. For instance, low H3K27me3 levels are linked to a poor prognosis for malignancies such as prostate and breast cancer.

4.3.2 Prognostic Biomarkers:

- **Histone Acetylation:** Aberrant methylation patterns, such as reduced H3K27me3 or enhanced H3K4me3, can be used to distinguish between malignant and normal tissues, which can help in the early detection of cancer [49]. For example, low H3K27me3 levels are associated with a poor prognosis for cancers including breast and prostate cancer.
- **Histone Phosphorylation:** Phosphorylation of H3S10 is linked to cell division. High proliferative tumors frequently have elevated levels of this marker, which can be utilized for predicting tumor aggressiveness and patient survival rates.

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4.3.3 Therapeutic Targets:

- **Histone Deacetylase Inhibitors (HDACi):** Tumor suppressor genes can be reactivated by medications that target HDACs and return acetylation levels to normal. FDA-approved for the treatment of specific lymphomas, HDAC inhibitors such as vorinostat and romidepsin are also being studied for additional cancers [51].
- **Methyltransferase Inhibitors:** Histone methyltransferases that catalyze H3K27 methylation, including EZH2, can be targeted to fix aberrant gene silence in malignancies. Clinical trials using EZH2 inhibitors are presently being conducted to treat malignancies such as B-cell lymphomas.

4.3.4 Predictive Biomarkers for Treatment Response:

- **Histone Ubiquitination:** The extent to which a patient will respond to chemotherapy can be predicted based on their level of histone ubiquitination, specifically H2Bub1. For example, loss of H2Bub1 influences the choice of alternative treatments because it is linked to resistance to specific chemotherapeutic drugs.

4.3.5 Monitoring Treatment Efficacy:

- **Epigenetic Biomarker Panels:** Histone modification marker panels provide a non-invasive way to assess patient reactions to medication and modify therapies when required [53]. They can also be used to monitor illness progression and treatment efficacy.

5. Non-Coding RNAs in Cancer Epigenetics

Francis Crick first put out the basic dogma of biology, which held that RNA molecules were just messengers between proteins and DNA [54]. Less than 2% of the human genome is known to encode proteins, with the remaining DNA traditionally thought to be "junk" DNA [55]. New studies have demonstrated a substantial correlation between biological and evolutionary complexity and the non-coding sections of the genome; big introns are more active in the nervous system and less active in cancer, indicating a regulatory function in tissue-specific gene expression [56]. A large portion of the genome is known to be actively transcribed under certain circumstances [57], containing genetic material that can either code for proteins or produce regulatory RNA transcripts that affect other genes [58]. Moreover, a single DNA sequence can have two purposes [59], controlling one transcript while encoding another protein. Non-coding RNAs, including as sncRNAs and lncRNAs, are essential to these activities [60], despite the fact that their length does not correspond to their location or function [61].

5.1 Overview of non-coding RNAs (e.g. miRNAs, lncRNAs)

Oncogenesis is assisted by alterations in both the genome and the epigenome. The second ones direct the transcriptional control of the former, whereas the former are linked to the activation of oncogenes and the inactivation of tumor suppressor genes. Accordingly, it is impossible to properly explain the processes leading to tumor development without acknowledging the prevalence of Bepimutations, which include abnormal histone modifications and DNA hyper- and hypo-methylation events throughout the whole genome [62]. CpG hypo methylation is linked to a particular chromatin conformation that permits transcription factors to access genetic material. In leukemia, they encourage the production of oncogenes such BCL2 [63]. As an alternative, CpG hypermethylation causes significant tumor suppressor genes, like BRCA1, to be down regulated in breast cancer [64]. The understanding that ncRNAs and epigenetics are essential to understanding the complete tumor genesis process challenges the historical understanding of cancer, which was centered on genetics and protein-coding genes.

In this case, ncRNAs have the ability to shape the epigenetic landscape of a normal or malignant cell and can be transcriptionally regulated by epigenetics. Numerous investigations shown that oncogenic and tumor suppressor non-coding RNAs are epigenetically dysregulated in cancer [65]. A typical characteristic of cancer cells is genome-wide hypo methylation of DNA, which also affects the genomic loci of non-coding RNAs [66]. Most frequently, research has examined the possibility of a local CGI hypermethylation-mediated transcriptional suppression. A cancer cell's complexity is now being revealed by the identification and examination of epigenetic pathways that are changed throughout tumor growth and metastasis. These pathways may also serve as indicators for diagnosis, prognosis, and targets for more effective treatments.

ncRNA	Expression	Epigenetic marks	Cancer type
miRNAs let-7a-3	Down regulation	Super-enhancer hyper methylation	Lung, breast [67]
miR-1	Down regulation	CGI hyper methylation ⇩H3K4me3	HCC [68]
miR-106b	Up regulation	Promoter hypo methylation	Colorectal [69]
miR-1224	Down regulation	CGI/CpG shores hyper methylation H3K9me3	Colorectal [70]
miR-124a	Down regulation	CGI hyper methylation	HCC [71]
miR-132	Down regulation	CGI hyper methylation⇧H3K 27me3	Bladder [72]
miR-133a-2	Down regulation	CGI hyper methylation	Colorectal, among others [73]
miR-145	Down regulation	CGI hyper methylation	HCC [74]

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miR-148a	Down regulation	CGI hyper methylation	Cervical [75]
miR-191	Up regulation	CGI hyper methylation	Breast [76]
miR-195	Down regulation	CGI hyper methylation	Pancreatic [78]
miR-200 loci	Down regulation	CGI hyper methylation	Uveal melanoma [79]
miR-203	Down regulation	CGI hyper methylation	HCC [80]
miR-212	Down regulation	CGI hyper methylation	Ovarian [81]
miR-23a	Up regulation	H3K27me3	HCC [82]
miR-25	Up regulation	CGI hypo methylation	HCC [83]
miR-27a	Up regulation	Promoter hypo methylation	HCC [84]
miR-34b/c	Down regulation	CGI hypo methylation	HCC [85]
miR-375	Down regulation	CGI hypo methylation	Colorectal [86]
miR-378	Down regulation	↓H3K4me3	HCC [87]

5.2. Role of non-coding RNAs in gene regulation

MiRNAs, or tiny non-coding RNAs, are around 22 nucleotides long and, by binding to the 3′ untranslated region (3′UTR) of target mRNAs, play a critical role in posttranscriptional gene silencing [88]. The degree to which they complementarity with the target determines whether they can cause mRNA degradation or restrict translation. Since perfect complementarity causes siRNAs, another type of short RNAs, to arise from lengthy double-stranded RNA precursors and frequently cause mRNA destruction, miRNAs usually cause translational repression. Small nucleolar RNAs (snoRNAs) and PIWI-interacting RNAs (piRNAs) also support posttranscriptional modifications and genome integrity, respectively. Circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) both regulate transcription and translation; because of their stability and patterns of expression, circRNAs have the potential to be biomarkers.

Non-coding RNAs (ncRNAs) have important roles in chromatin remodeling and epigenetic control. For instance, chromatin regulatory complexes can be directed by nuclear long noncoding RNAs to particular genomic loci, so affecting the silencing or activation of transcription. Across vast genomic areas, transcriptional repression is mediated by the well-known lncRNA HOTAIR in conjunction with chromatin modifiers [89]. Another lncRNA, Xist, is controlled by other lncRNAs and epigenetic processes and is essential for X-chromosome inactivation in females [93]. These many kinds of non-coding RNAs play a crucial role in both healthy and pathological conditions in gene expression and cellular function.

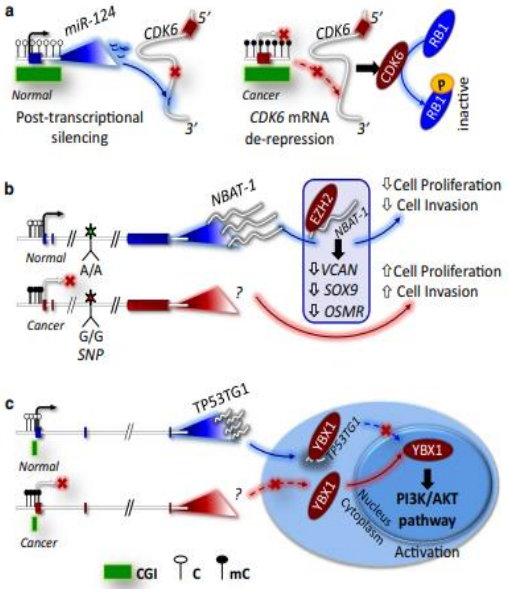

Fig 8.Epigenetic regulation of ncRNAs in cancer [90]

5.3. Cancer-specific non-coding RNA changes

Only around 3% of the human genome gets transcribed into mRNAs, which code for proteins. Approximately 75% of the human genome is transcribed into RNA. Non-coding RNAs (ncRNAs) are classified according to their length, shape, and location. There are numerous major forms of ncRNAs, each having a specific function in cancer, including microRNA (miRNA), long ncRNA (lncRNA), circular RNA (circRNA), and PIWI-interacting RNA (piRNA). miRNAs are short RNAs, around 22 nucleotides in length, that attach to target mRNAs' complementary sequences to cause RNA-induced silencing complex (RISC)-mediated degradation of the target mRNAs. piRNAs are mostly present in germline cells, where they bind to PIWI proteins and take role in chromatin epigenetic regulation. They are typically 24–30 nucleotides long, and they were initially discovered in Drosophila [91].

Both circRNAs and lncRNAs are longer than 200 nucleotides; circRNAs create structures resembling rings, whereas lncRNAs are linear. Exons, introns, intergenic regions, or 5′/3′ untranslated regions can all be used for the transcription of either type, which then folds into intricate secondary structures that allow for interactions with proteins, RNA, and DNA. Through a variety of strategies, including functioning as miRNA decoys to stop mRNA degradation, modifying transcription factor binding to promoters, and acting as scaffolds for downstream signaling cascades and protein-protein interactions, these ncRNAs control the expression of genes [92]. Additionally, recent research indicates that circRNAs and lncRNAs may play a role in the epigenetic modification of chromatin, which in turn affects gene expression.

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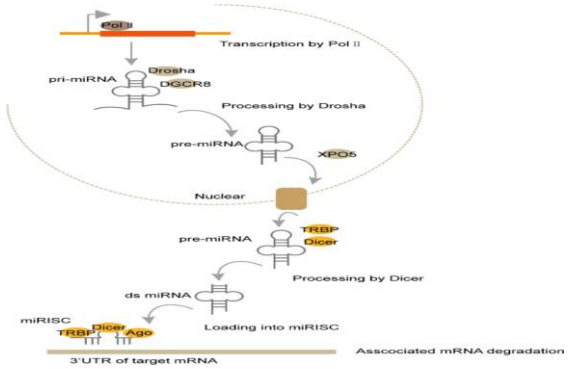


Fig 9.The biogenesis and effector machineries of miRNAs [93]

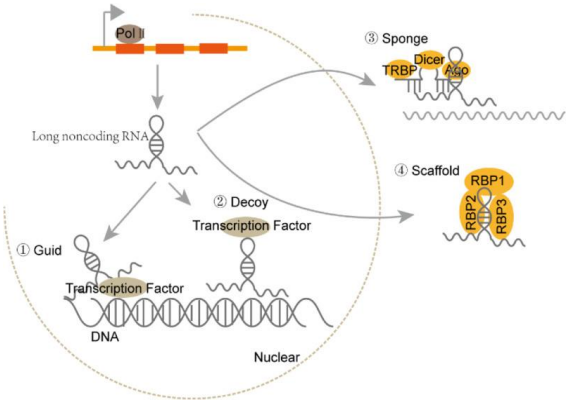
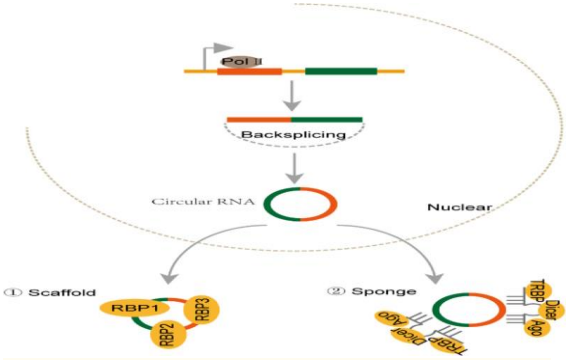


Fig 10.**The biogenesis and effector machineries of lncRNAs** [94]



The biogenesis and effector machineries of circRNAs [95]

5.4. Clinical applications of non-coding RNA markers

Long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs) are examples of non-coding RNAs (ncRNAs) that play important roles in gene regulation and are becoming more and more understood to be potential clinical biomarkers and therapeutic targets. They are perfect candidates for non-invasive diagnostic techniques because of their stability in bodily fluids and specificity in disease conditions.

- **MicroRNAs as Diagnostic and Prognostic Biomarkers**
  Recent research has brought attention to the function of miRNAs as biomarkers in a variety of malignancies. For example, miR-21 has been found to be a possible biomarker for both prognosis and diagnosis in a number of cancers, such as colorectal, lung, and breast cancer. Dysregulation of miR-21 is associated with a poor prognosis and treatment resistance. [96]
- **Long Non-Coding RNAs in Cancer Therapy**
- It has been demonstrated that lncRNAs like MALAT1 and HOTAIR have a role in the development and spread of cancer. The epithelial-mesenchymal transition (EMT) pathway has been linked to MALAT1, in particular, and preclinical models have demonstrated the potential of targeting MALAT1 with certain inhibitors to reduce tumor metastasis [97].
- **Circular RNAs as Novel Biomarkers**
- CircRNAs are great candidates for biomarkers because of their covalently closed loop designs, which show remarkable stability in bodily fluids. CircRNA ciRS-7 was found to be a promising biomarker for hepatocellular carcinoma early detection in a study by [98], showcasing its excellent sensitivity and specificity in differentiating between cancer patients and healthy individuals.

Conclusion

In conclusion, epigenetic modifications are essential to the onset and spread of cancer because they affect vital functions like apoptosis, cell cycle regulation, and gene expression. Gaining knowledge about the processes underlying DNA methylation, histone modification, chromatin remodeling, and non-coding RNA participation will help to better understand the intricate interactions between epigenetics and cancer. More efficient and individualized cancer treatments are possible thanks to the potential of epigenetic biomarkers in cancer diagnosis and prognosis as well as the development of epigenetic therapeutics. Research is opening up new possibilities for enhancing cancer outcomes and expanding our understanding of cancer biology as it continues to combine epigenetic data with genomic and transcriptome information.

References

1. Waddington, C. H. (1942). The epigenotype. *Endeavour*, *1*, 18-20
2. Waddington, C. H. (1968). Towards a theoretical biology. *Nature*, *218*(5141), 525-527.
3. Wu, C. T., & Morris, J. R. (2001). Genes, genetics, and epigenetics: a correspondence. *Science*, *293*(5532), 1103-1105.
4. Ilango, S., Paital, B., Jayachandran, P., Padma, P. R., & Nirmaladevi, R. (2020). Epigenetic alterations in cancer. *Frontiers in Bioscience-Landmark*, *25*(6), 1058-1109.
5. Sandler I, Sandler L. A conceptual ambiguity that contributed to the neglect of Mendel's paper. History Phil Life Sciences 1985; 7:3-70.

*Corresponding Author: **Rida Fatima**

6. Waddington CH. Introduction to Modern Genetics. London: Allen and Unwin 1939.
7. Hadorn E. Developmental Genetics and Lethal Factors. 1960:Methuen, London. First published in German, 1955:G.T. Verlag, Stuttgart
8. Deng, D., Liu, Z., & Du, Y. (2010). Epigenetic alterations as cancer diagnostic, prognostic, and predictive biomarkers. *Advances in genetics*, *71*, 125-176.
9. Craig, J. M., & Bickmore, W. A. (1994). The distribution of CpG islands in mammalian chromosomes. *Nature genetics*, *7*(3), 376-382.
10. Klutstein, M., Nejman, D., Greenfield, R., & Cedar, H. (2016). DNA methylation in cancer and aging. *Cancer research*, *76*(12), 3446-3450.
11. Luczak MW, Jagodziński PP. The role of DNA methylation in cancer development. Folia Histochem Cytobiol. 2006;44(3):143- 154
12. Klutstein M, Nejman D, Greenfield R, Cedar H. DNA methylation in cancer and aging. Cancer Res. 2016;76(12):3446-3450. doi:10.1158/0008-5472.CAN-15-3278
13. Craig JM, Bickmore WA. The distribution of CpG islands in mammalian chromosomes. Nat Genet. 1994;7(3):376-382. doi:10. 1038/ng0794-376
14. Takai, D., & Jones, P. A. (2002). Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proceedings of the national academy of sciences*, *99*(6), 3740-3745.
15. Saxonov, S., Berg, P., & Brutlag, D. L. (2006). A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proceedings of the National Academy of Sciences*, *103*(5), 1412-1417.
16. Kiselev, I. S., Kulakova, O. G., Boyko, A. N., & Favorova, O. O. (2021). DNA methylation as an epigenetic mechanism in the development of multiple sclerosis. *Acta Naturae*, *13*(2), 45.
17. Bestor TH (2000) The DNA methyltransferases of mammals. Hum Mol Genet 9: 2395–2402
18. Rhee I et al. (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 416: 552–556
19. Fuks F et al. (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat Genet 24: 88–91
20. Jenuwein T and Allis CD (2001) Translating the histone code. Science 293: 1074–1080
21. Baylin, S. B. (2005). DNA methylation and gene silencing in cancer. *Nature clinical practice Oncology*, *2*(Suppl 1), S4-S11.
22. Hinoue, T., Weisenberger, D. J., Lange, C. P., Shen, H., Byun, H. M., Van Den Berg, D., ... & Laird, P. W. (2012). Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome research*, *22*(2), 271-282.
23. Pfeifer, G. P. (2018). Defining driver DNA methylation changes in human cancer. *International journal of molecular sciences*, *19*(4), 1166.
24. Baylin, S. B., & Jones, P. A. (2016). Epigenetic determinants of cancer. *Cold Spring Harbor Perspectives in Biology*, *8*(9), a019505.
25. Esteller, M. (2008). Epigenetics in cancer. *The New England Journal of Medicine*, *358*(11), 1148-1159.
26. Herman, J. G., & Baylin, S. B. (2003). Gene silencing in cancer in association with promoter hypermethylation. *The New England Journal of Medicine*, *349*(21), 2042-2054.
27. Jones, P. A., & Baylin, S. B. (2007). The epigenomics of cancer. *Cell*, *128*(4), 683-692.
28. Sandoval, J., & Esteller, M. (2012). Cancer epigenomics: Beyond genomics. *Current Opinion in Genetics & Development*, *22*(1), 50-55.
29. Shen, H., & Laird, P. W. (2013). Interplay between the cancer genome and epigenome. *Cell*, *153*(1), 38-55. a
30. Zhao Z, Shilatifard A. Epigenetic modifications of histones in cancer. Genome Biol. 2019;20(1):1–16. doi:10.1186/s13059-019-1870-5.
31. Chan JC, Maze I. Nothing is yet set in (hi)stone: novel post-translational modifications regulating chromatin function. Trends Biochem Sci. 2020;45(10):829–844. doi:10.1016/j.tibs.2020.05.009
32. Saleh R, Toor SM, Sasidharan Nair V, et al. Role of epigenetic modifications in inhibitory immune checkpoints in cancer development and progression. Front Immunol. 2020;11:1469. doi:10.3389/fimmu.2020.01469
33. Xhemalce B, Dawson MA, Bannister AJ. In Reviews in Cell Biology and Molecular Medicine. Histone modifications; 2011.
34. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell. 2012;150(1):12–27. doi:10.1016/j.cell.2012.06.013.
35. Sharma A, Liu H, Herwig-Carl MC, et al. Epigenetic regulatory enzymes: mutation prevalence and coexistence in cancers. Cancer Investig. 2021;39(3):257–273. doi:10.1080/07357907.2021.1872593.
36. Mohammad F, Helin K. Oncohistones: drivers of pediatric cancers. Genes Dev. 2017;31(23-24):2313–2324. doi:10.1101/gad.309013.
37. Choudhary C, Kumar C, Gnad F, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009;325(5942):834–840. doi:10.1126/science.1175371.
38. Martin BJE, Brind'Amour J, Kuzmin A, et al. Transcription shapes genome-wide histone acetylation patterns. Nat Commun. 2021;12(1):210. doi:10.1038/s41467-020-20543-z.
39. Kori Y, Sidoli S, Yuan ZF, et al. Proteome-wide acetylation dynamics in human cells. Sci Rep.

2017;7(1):10296. doi:10.1038/s41598-017-09918-3.
40. Cohen I, Poreba E, Kamieniarz K, et al. Histone modifiers in cancer: friends or foes? Genes Cancer. 2011;2(6):631–647. doi:10.1177/1947601911417176.
41. Sugiura M, Sato H, Kanesaka M, et al. Epigenetic modifications in prostate cancer. Int J Urol. 2021;28:140–149. doi:10.1111/iju.14406.
42. Zhan X, Guo S, Li Y, et al. Glioma stem-like cells evade interferon suppression through MBD3/NuRD complex-mediated STAT1 downregulation. J Exp Med. 2020;4(5):e20191340 217. doi:10.1084/jem.20191340.
43. Jones PA, Issa JP, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016;17(10):630–641. doi:10.1038/nrg.2016.93.
44. Villanueva L, Álvarez-Errico D, Esteller M. The contribution of epigenetics to cancer immunotherapy. Trends Immunol. 2020;41(8):676–691. doi:10.1016/j.it.2020.06.002.
45. Surapaneni SK, Bhat ZR, Tikoo K. MicroRNA-941 regulates the proliferation of breast cancer cells by altering histone H3 Ser 10 phosphorylation. Sci Rep. 2020;10(1):17954. doi:10.1038/s41598-020-74847-7.
46. Komar D, Juszczynski P. Rebelled epigenome: histone H3S10 phosphorylation and H3S10 kinases in cancer biology and therapy. Clin Epigenetics. 2020;12(1):147. doi:10.1186/s13148-020-00941-2.
47. Zhao, Z., & Shilatifard, A. (2019). Epigenetic modifications of histones in cancer. *Genome biology*, *20*, 1-16.
48. Chan, J. C., & Maze, I. (2020). Nothing is yet set in (hi) stone: novel post-translational modifications regulating chromatin function. *Trends in biochemical sciences*, *45*(10), 829-844.
49. Yang, Y., Zhang, M., & Wang, Y. (2022). The roles of histone modifications in tumorigenesis and associated inhibitors in cancer therapy. *Journal of the National Cancer Center*, *2*(4), 277-290.
50. Dawson, M. A., & Kouzarides, T. (2012). Cancer epigenetics: From mechanism to therapy. *Cell*, 150(1), 12-27.
51. Seligson, D. B., Horvath, S., McBrian, M. A., Mah, V., Yu, H., Tze, S., ... & Kurdistani, S. K. (2005). Global histone modification patterns predict risk of prostate cancer recurrence. *Nature*, 435(7046), 1262-1266.
52. Helin, K., & Dhanak, D. (2013). Chromatin proteins and modifications as drug targets. *Nature*, 502(7472), 480-488.
53. Chervona, Y., & Costa, M. (2012). Histone modifications and cancer: Biomarkers of prognosis? *American Journal of Cancer Research*, 2(5), 589-597.
54. Crick, F. H. (1958). On protein synthesis. Symposia of the Society for Experimental Biology, 12, 138–163
55. Alexander, R. P., Fang, G., Rozowsky, J., Snyder, M., & Gerstein, M. B. (2010). Annotating non-coding regions of the genome. Nature Reviews. Genetics, 11(8), 559–571.
56. Taft, R. J., Pheasant, M., & Mattick, J. S. (2007). The relationship between non-protein-coding DNA and eukaryotic complexity. BioEssays, 29(3), 288–299.
57. ENCODE. (2012). Project Consortium, an integrated encyclopedia of DNA elements in the human genome. Nature, 489(7414), 57–74.
58. Dhanasekaran, K., Kumari, S., & Kanduri, C. (2013). Noncoding RNAs in chromatin organization and transcription regulation: an epigenetic view. Sub-Cellular Biochemistry, 61, 343–372
59. Tay, Y., Kats, L., Salmena, L., Weiss, D., Tan, S. M., Ala, U., Karreth, F., Poliseno, L., Provero, P., Di Cunto, F., Lieberman, J., Rigoutsos, I., & Pandolfi, P. P. (2011). Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell, 147(2), 344–357.
60. Kapranov, P., Cheng, J., Dike, S., Nix, D. A., Duttagupta, R., Willingham, A. T., Stadler, P. F., Hertel, J., Hackermuller, J., Hofacker, I. L., Bell, I., Cheung, E., Drenkow, J., Dumais, E., Patel, S., Helt, G., Ganesh, M., Ghosh, S., Piccolboni, A., Sementchenko, V., Tammana, H., & Gingeras, T. R. (2007). RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science, 316(5830), 1484–1488.
61. Walsh, C. P., Chaillet, J. R., & Bestor, T. H. (1998). Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. Nature Genetics, 20(2), 116–117.
62. Oey, H., & Whitelaw, E. (2014). On the meaning of the word 'epimutation'. Trends in Genetics, 30(12), 519–520.
63. Hanada, M., Delia, D., Aiello, A., Stadtmauer, E., & Reed, J. C. (1993). bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. Blood, 82(6), 1820–1828
64. Esteller, M., Silva, J. M., Dominguez, G., Bonilla, F., MatiasGuiu, X., Lerma, E., Bussaglia, E., Prat, J., Harkes, I. C., Repasky, E. A., Gabrielson, E., Schutte, M., Baylin, S. B., & Herman, J. G. (2000). Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. Journal of the National Cancer Institute, 92(7), 564–569.
65. Heyn, H., Vidal, E., Ferreira, H. J., Vizoso, M., Sayols, S., Gomez, A., Moran, S., Boque-Sastre, R., Guil, S., Martinez-Cardus, A., Lin, C. Y., Royo, R., Sanchez-Mut, J. V., Martinez, R., Gut, M.,

Torrents, D., Orozco, M., Gut, I., Young, R. A., & Esteller, M. (2016). Epigenomic analysis detects aberrant super-enhancer DNA methylation in human cancer. Genome Biology, 17, 11

66. Datta, J., Kutay, H., Nasser, M. W., Nuovo, G. J., Wang, B., Majumder, S., Liu, C. G., Volinia, S., Croce, C. M., Schmittgen, T. D., Ghoshal, K., & Jacob, S. T. (2008). Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Research, 68(13), 50
67. Heyn, H., Vidal, E., Ferreira, H. J., Vizoso, M., Sayols, S., Gomez, A., Moran, S., Boque-Sastre, R., Guil, S., Martinez-Cardus, A., Lin, C. Y., Royo, R., Sanchez-Mut, J. V., Martinez, R., Gut, M., Torrents, D., Orozco, M., Gut, I., Young, R. A., & Esteller, M. (2016). Epigenomic analysis detects aberrant super-enhancer DNA methylation in human cancer. Genome Biology, 17, 11.
68. Datta, J., Kutay, H., Nasser, M. W., Nuovo, G. J., Wang, B., Majumder, S., Liu, C. G., Volinia, S., Croce, C. M., Schmittgen, T. D., Ghoshal, K., & Jacob, S. T. (2008). Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Research, 68(13), 5049–5058
69. Suzuki, H., Takatsuka, S., Akashi, H., Yamamoto, E., Nojima, M., Maruyama, R., Kai, M., Yamano, H. O., Sasaki, Y., Tokino, T., Shinomura, Y., Imai, K., & Toyota, M. (2011). Genome-wide profiling of chromatin signatures reveals epigenetic regulation of microRNA genes in colorectal cancer. Cancer Research, 71(17), 5646–5658.
70. Chen, W. S., Leung, C. M., Pan, H. W., Hu, L. Y., Li, S. C., Ho, M. R., & Tsai, K. W. (2012). Silencing of miR-1-1 and miR-133a-2 cluster expression by DNA hypermethylation in colorectal cancer. Oncology Reports, 28(3), 1069–1076.
71. He, X. X., Kuang, S. Z., Liao, J. Z., Xu, C. R., Chang, Y., Wu, Y. L., Gong, J., Tian, D. A., Guo, A. Y., & Lin, J. S. (2015). The regulation of microRNA expression by DNA methylation in hepatocellular carcinoma. Molecular BioSystems, 11((2), 532–539.
72. Dudziec, E., Miah, S., Choudhry, H. M., Owen, H. C., Blizard, S., Glove r, M., Hamdy, F. C., & Catto, J. W. (2011). Hypermethylation of CpG islands and shores around specific microRNAs and mirtrons is associated with the phenotype and presence of bladder cancer. Clinical Cancer Research, 17(6), 1287–1296.
73. Lujambio, A., Ropero, S., Ballestar, E., Fraga, M. F., Cerrato, C., Setien, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Git, A., Spiteri, I., Das, P. P., Caldas, C., Miska, E., & Esteller, M. (2007). Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Research, 67(4), 1424–1429.
74. Furuta, M., Kozaki, K. I., Tanaka, S., Arii, S., Imoto, I., & Inazawa, J. (2010). miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis, 31(5), 766–776.
75. Wilting, S. M., van Boerdonk, R. A., Henken, F. E., Meijer, C. J., Diosdado, B., Meijer, G. A., le Sage, C., Agami, R., Snijders, P. J., & Steenbergen, R. D. (2010). Methylation-mediated silencing and tumour suppressive function of hsa-miR-124 in cervical cancer. Molecular Cancer, 9, 167.
76. Lv, X. B., Jiao, Y., Qing, Y., Hu, H., Cui, X., Lin, T., Song, E., & Yu, F. (2011). miR-124 suppresses multiple steps of breast cancer metastasis by targeting a cohort of pro-metastatic genes in vitro. Chinese Journal of Cancer, 30(12), 821–830.
77. Wang, P., Chen, L., Zhang, J., Chen, H., Fan, J., Wang, K., Luo, J., Chen, Z., Meng, Z., & Liu, L. (2014). Methylation-mediated silencing of the miR-124 genes facilitates pancreatic cancer progression and metastasis by targeting Rac1. Oncogene, 33(4), 514–524.
78. Chen, X., He, D., Dong, X. D., Dong, F., Wang, J., Wang, L., Tang, J., Hu, D. N., Yan, D., & Tu, L. (2013). MicroRNA-124a is epigenetically regulated and acts as a tumor suppressor by controlling multiple targets in uveal melanoma. Investigative Ophthalmology & Visual Science, 54(3), 2248–2256.
79. Tivnan, A., Zhao, J., Johns, T. G., Day, B. W., Stringer, B. W., Boyd, A. W., Tiwari, S., Giles, K. M., Teo, C., & McDonald, K. L. (2014). The tumor suppressor microRNA, miR-124a, is regulated by epigenetic silencing and by the transcriptional factor, REST in glioblastoma. Tumour Biology, 35(2), 1459–1465.
80. Formosa, A., Lena, A. M., Markert, E. K., Cortelli, S., Miano, R., Mauriello, A., Croce, N., Vandesompele, J., Mestdagh, P., FinazziAgro, E., Levine, A. J., Melino, G., Bernardini, S., & Candi, E. (2013). DNA methylation silences miR-132 in prostate cancer. Oncogene, 32(1), 127–134.
81. Qin, J., Ke, J., Xu, J., Wang, F., Zhou, Y., Jiang, Y., & Wang, Z. (2015). Downregulation of microRNA-132 by DNA hypermethylation is associated with cell invasion in colorectal cancer. Onco Targets Ther, 8, 3639–3648.
82. Zhang, S., Hao, J., Xie, F., Hu, X., Liu, C., Tong, J., Zhou, J., Wu, J., & Shao, C. (2011). Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development. Carcinogenesis, 32(8), 1183–1189.
83. Lin L, Wang Z, Jin H, Shi H, Lu Z, Qi Z (2016) MiR-212/132 is epigenetically downregulated by SOX4/EZH2-H3K27me3 feedback loop in ovarian cancer cells. Tumour Biol. 37(12), 15719– 15727.
84. Rani, S. B., Rathod, S. S., Karthik, S., Kaur, N., Muzumdar, D., & Shiras, A. S. (2013). MiR-145

functions as a tumor-suppressive RNA by targeting Sox9 and adducin 3 in human glioma cells. Neuro-Oncology, 15(10), 1302–1316.

85. He, Y., Cui, Y., Wang, W., Gu, J., Guo, S., Ma, K., & Luo, X. (2011). Hypomethylation of the hsa-miR-191 locus causes high expression of hsa-mir-191 and promotes the epithelial-to-mesenchymal transition in hepatocellular carcinoma. Neoplasia, 13(9), 841–853.
86. Davalos, V., Moutinho, C., Villanueva, A., Boque, R., Silva, P., Carneiro, F., & Esteller, M. (2012). Dynamic epigenetic regulation of the microRNA-200 family mediates epithelial and mesenchymal transitions in human tumorigenesis. Oncogene, 31(16), 2062–2074.
87. Toyota, M., Suzuki, H., Sasaki, Y., Maruyama, R., Imai, K., Shinomura, Y., & Tokino, T. (2008). Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Research, 68(11), 4123–4132.
88. Kimura, T. (2020). Non-coding natural antisense RNA: mechanisms of action in the regulation of target gene expression and its clinical implications. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*, *140*(5), 687-700.
89. Vos, P. D., Leedman, P. J., Filipovska, A., & Rackham, O. (2019). Modulation of miRNA function by natural and synthetic RNA-binding proteins in cancer. *Cellular and Molecular Life Sciences*, *76*(19), 3745-3752.
90. Zeng, Q., Wan, H., Zhao, S., Xu, H., Tang, T., Oware, K. A., & Qu, S. (2020). Role of PIWI-interacting RNAS on cell survival: Proliferation, apoptosis, and cycle. *IUBMB life*, *72*(9), 1870-1878.
91. Wang, N., Yu, Y., Xu, B., Zhang, M., Li, Q., & Miao, L. (2019). Pivotal prognostic and diagnostic role of the long non-coding RNA colon cancer-associated transcript 1 expression in human cancer. *Molecular Medicine Reports*, *19*(2), 771-782.
92. Zhao, W., An, Y., Liang, Y., & Xie, X. W. (2014). Role of HOTAIR long noncoding RNA in metastatic progression of lung cancer. *Eur Rev Med Pharmacol Sci*, *18*(13), 1930-1936.
93. Lujambio, A., Ropero, S., Ballestar, E., Fraga, M. F., Cerrato, C., Setien, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Git, A., Spiteri, I., Das, P. P., Caldas, C., Miska, E., & Esteller, M. (2007). Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Research, 67(4), 1424–1429.
94. Furuta, M., Kozaki, K. I., Tanaka, S., Arii, S., Imoto, I., & Inazawa, J. (2010). miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis, 31(5), 766–77
95. Yan, H., & Bu, P. (2021). Non-coding RNA in cancer. *Essays in biochemistry*, *65*(4), 625-639.
96. Yan, H., & Bu, P. (2021). Non-coding RNA in cancer. *Essays in biochemistry*, *65*(4), 625-639.
97. Ebrahimi, F., Gopalan, V., Wahab, R., Lu, C. T., Smith, R. A., & Lam, A. K. Y. (2015). Deregulation of miR-126 expression in colorectal cancer pathogenesis and its clinical significance. *Experimental cell research*, *339*(2), 333-341.
98. Lechman, E. R., Gentner, B., Ng, S. W., Schoof, E. M., van Galen, P., Kennedy, J. A., ... & Dick, J. E. (2016). miR-126 regulates distinct self-renewal outcomes in normal and malignant hematopoietic stem cells. *Cancer cell*, *29*(2), 214-228.
99. Hu, J., & Wang, X. F. (2016). HIF-miR-215-KDM1B promotes glioma-initiating cell adaptation to hypoxia. *Cell Cycle*, *15*(15), 1939-1940.

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