

## Review Article

# An Overview of the Epigenetic Modifications of Gene Expression in Tumorigenesis

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### Abstract

The five leading causes of cancer-related deaths are lung (1,760,000 deaths), colorectal (862,000 deaths), stomach (783,000 deaths), liver (782,000 deaths), and breast (627,000 deaths) cancers. Epigenetic changes can alter chromatin compaction, leading to the regulation of gene expression without changing the primary DNA sequence. Epigenetic mechanisms are normally involved in cellular processes such as genomic stability, chromosome X inactivation, and embryonic development and differentiation. Similar to other types of chromatin modifications, DNA methylation has been verified to affect the expression of various genes. Any impairment in these mechanisms alters the regulation of gene expression and can contribute to malignant cell transformation. Over the past few years, extensive innovations within the field of epigenetics have encouraged its application as a major strategy for the treatment of important diseases such as cancer.

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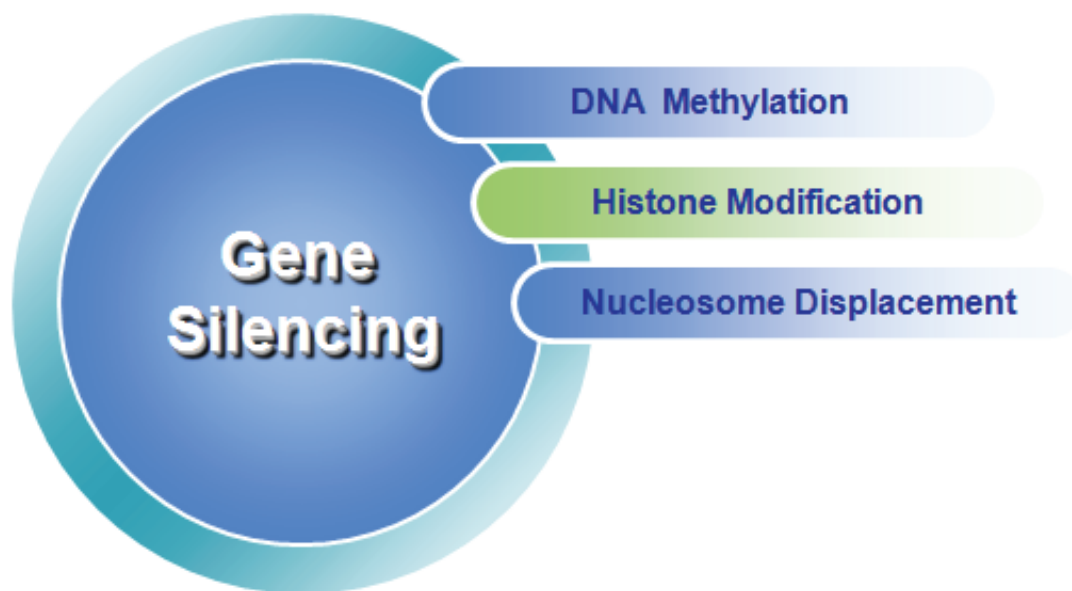
## 1. Epigenetics; a Viewpoint on Gene Expression

Genetic mutations and epigenetic alterations both contribute to tumorigenesis (1). The term “epigenetics” was initially introduced in 1942 by Canard Wadding via combining the two words “epigenesis and genetic”. The Greek prefix *epi-* in “epigenetics” means “trans”. Epigenetics is defined as hereditary changes in gene function without any accompanying change in the nucleotide sequence of DNA (2). While the total amount of DNA within the genome remains constant throughout cell differentiation and specialization, DNA expression profiles vary widely among different cell types and during various growth stages (1, 3). The main cause of alterations in developmental gene expression is epigenetic changes that are stably inheritable despite the fact that they do not alter nucleotide sequences (4). These epigenetic modifications include:

1. DNA methylation
2. Histone modifications

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- (a) Histone chemical changes
    - Acetylation and deacetylation
    - Methylation and demethylation
    - Phosphorylation, ubiquitylation, and sumoylation
  - (b) Nucleosome replacement
3. Regulatory microRNA (miRNA) (1, 5)



**Figure 1:** Hereditary of gene silencing is regulated by mechanisms that include DNA methylation, histone modification, and nucleosome displacement. DNA methyltransferase (DNMT), histone deacetylase (HDAC), histone methyltransferases (HMTs), and nucleosomal alteration factors (NURFs) are involved in DNA modifications and epigenetic regulation. Collectively, they trigger an inhibitory effect that results in gene silencing. The expression of certain genes can be physiologically omitted at a given time to facilitate the development and evolution of organisms; however, certain diseases such as cancer may occur as a result of pathological gene silencing.

## 2. DNA Methylation

In mammals, DNA methylation occurs mainly at the C5 of cytosine (C) bases located in CpG dinucleotides. These specific dinucleotides are mainly concentrated in the regulatory regions of most genes and are known as CpG islands (6). Hypermethylation of the regulatory region typically suppresses the expression of tumor suppressor genes in neoplastic cells (7, 8, 10). 5-methylcytosine is considered to be a hot spot and target for exogenous and endogenous mutagens in different tumors (9). Several gene families involved in DNA repair, hormone receptor function, and angiogenesis inhibition are silenced as a result of DNA methylation. DNA methylation can alter gene expression via selective attachment of regulatory transcriptional proteins that are different from those bind to the non-methylated DNA. Hypermethylation-derived gene silencing that drives carcinogenesis can also provide a major target for the prevention and treatment

of cancer (12). It has been demonstrated that epigenetic modifications such as DNA methylation and histone modifications contribute to long-term gene silencing and carcinogenesis (13). For example, alterations in the methylation pattern of genes, particularly those involved in signaling pathways, are significantly correlated with the incidence of gastric cancer (14-17). The methylation of CpG dinucleotides in DNA plays an important role in the stability of chromosome structure and gene expression. Typically, DNA methylation within promoter regions prevents the attachment and activation of transcription complexes and causes gene silencing (18-22). Additionally, DNA methylation can trigger binding of other chromatin modifying proteins such as HDACs and histone-methyl transferases (HMTs), and this in turn results in the occurrence of further epigenetic modifications to the chromatin (23-25). Previous research indicates that the expression of certain genes such as P16, hmlh1, and timp3 are deeply suppressed in gastric carcinogenesis as a result of hypermethylation (26-28). Generally, the inactivation of these genes can result from genetic or epigenetic changes to both alleles. Hmlh1 and P16 genes are often deactivated by epigenetic modifications in sporadic gastric adenocarcinoma (29, 30). DAP kinase (Death Associated Protein Kinase), a serine-threonine kinase that induces apoptosis, has been observed to be inactivated due to methylation of CpG sites in breast, bladder, kidney and lung cancers and also malignant lymphocyte B cells (31-33). The expression of the THBS1 gene, an angiogenesis inhibitor, is decreased in different types of human tumors (34). Hypermethylation of the THBS1 promoter has been observed in several cancer cell lines and also in brain tumors (35). It was reported that hypermethylation and silencing of RUNX3 transcriptional factors is associated with a number of cancers, particularly gastric cancer. The monitoring of RUNX3 expression may be useful for assessing the occurrence of cancers (36). RASSF1A is another tumor suppressor factor that is silenced in gastric tumors and other malignancies via hypermethylation of the regulatory regions (14).

### 3. Histone Modifications and Alteration of Gene Expression

Chromatin is a combination of DNA and proteins that organize and stabilize the structure of DNA, the basic heredity material, and chromatin structure regulates the transcriptional pattern. The main subunit of chromatin, specifically the nucleosome, is a histone octamer that consists of four central histones (H2A, H2B, H3, and H4) that are arranged as two distinct dimers (H2A/H2B and H3/H4) encompassed by a 146bp of DNA (41, 42). Histone modifications influence the ability of DNA to bind to other proteins that affect chromatin compaction. The structure and organization of chromatin are two important factors that regulate gene expression. Both the location and the components of the nucleosome within a given promoter region control transcription levels, and these factors are regulated by intracellular and extracellular signals (37). The chromatin structure is largely influenced by the N-terminal region of histone proteins. Histone modifications such as methylation, phosphorylation, acetylation, ubiquitination, ADP ribosylation, proline deamination, and isomerization are considered to be the most important epigenetic modifications. These alterations generally occur at the N-terminal regions of histone

proteins, and these modifications play a significant role in gene expression alterations (38, 39). While histone acetylation weakens the association of histone proteins with DNA and positively affects transcription rate, histone methylation can either activate or deactivate gene transcription based on the specific amino acid residue that is methylated. For example, methylation of histone H3 lysine 4, 36, and 79 is associated with active transcription, while addition of a methyl group to histone H4 lysine 9 and 27 negatively affects gene transcription. The methyl transfer reaction is catalyzed by histone methyl transferase (HMTs) enzymes that act specifically for different substrates (40). Methylation of H3 Lysine 9 within the GKN1 promoter, a process involved in accurate function of the gastric mucosa, is an example of the role of epigenetic modifications in the induction of cancer cells (41). Although histone methylation was recognized as a distinct process regulating epigenetic modifications and it was believed that methylation independently regulates the structure of chromatin and gene expression (42-45), phosphorylation of H3 serine 10 is also defined as another type of epigenetic alteration that controls the structure of chromatin by preventing methylation of lysine (46). Poly-ADP ribosylation of histone proteins alters chromatin structure in two ways. First, short chains of ADP polymers are covalently added to histone proteins. Second, branched and long chain polymers in the PARP1 chain are attached to histones (47, 48). These histone modifications play an important role in the epigenetic regulation of corresponding genes. Acetylation and methylation are two important histone changes that can involve in tumorigenesis through epigenetic mechanisms. Acetyl and methyl residues are well-known as epigenetic markers in cancer studies (49, 50).

#### 4. Histone Acetylation and Cancer

Histone acetylation was first hypothesized by Vincent Allfrey, who suggested that acetylation is associated with gene transcription in eukaryotic cells (57). It is now established that histone acetylation is more specific than other histone modifications. In most cases, this epigenetic alteration occurs on the amine groups of the lysine residue. Transfer of the acetyl groups is mediated by histone acetyltransferase (HATs) and histone-deacetylase (HDACs) enzymes. The steady-state level of histone acetylation is achieved by the balanced activity of HAT and HDAC. Generally, increased levels of histone acetylation (hyperacetylation) would neutralize positive charge of histone tails and cause a reduction in DNA-histone binding affinity (52-55). Disruption of acetylation homeostasis is an important factor that regulates gene expression and can be associated with carcinogenesis (56). It appears that the acetylation of histones H3 and H4 is particularly important in the context of chromatin structure, translation, and expression (57).

The three main families of the HAT are described below.

1. MOZ / YBF2 / SAS2 / TIP60 / Myb
2. GCN5-N-acetyl transferase (GNAT)
3. CBP / P300 family.

HATs transfer an acetyl group into the lysine residues of the histone proteins (58, 59). The role of HAT enzymes in gene transcription, mutation and expression have been observed in various types of cancers. An imbalance between acetylation and deacetylation levels has been observed in many tumors. A decrease in histone acetylation is associated with reduced potential of tumor progression and metastasis. Trichostatin A (TSA) is a kind of histone deacetylation inhibitor that inhibits cancer cells invasion and induces apoptosis by increasing histone acetylation, particularly within gene promoter regions. The use of TSA as a cancer therapeutic has recently been explored. Gene expression can be altered in metastatic tumors by histone deacetylation, and therefore, histone acetylation may provide a target for cancer treatment in metastatic stages or at early stages (60). Histone acetyltransferases co-regulate gene expression by binding to transcription factors. Additionally, the acetylation of non-histone proteins such as PCAF, P300 and CBP by histone acetyltransferases can result in oncogenic transformation (58, 61, 62). Roles for histone acetyltransferases have been reported in both liver and solid cancers. It was observed that P300 mutation is associated with solid tumor formation in the intestine, stomach, chest, and pancreas (62, 63). Tip60 histone acetyltransferase is involved in tumorigenesis pathways via the induction of transcriptional changes in *P53* and *Myc* genes (64). Specifically, the acetylation pattern of the *P53* gene promoter is altered by Tip60 and this results in release of cells from the G0 stage and subsequent apoptosis (65, 66). Decreasing the expression of Tip60 reduces P53 acetylation and apoptotic signaling, and this decrease in expression increases the malignant potential of tumor cells. Due to the role of Tip60 in tumor suppression, even the rare loss of a single allele is correlated with malignancies such as lymphoma, ovarian, and head and neck tumors (64).

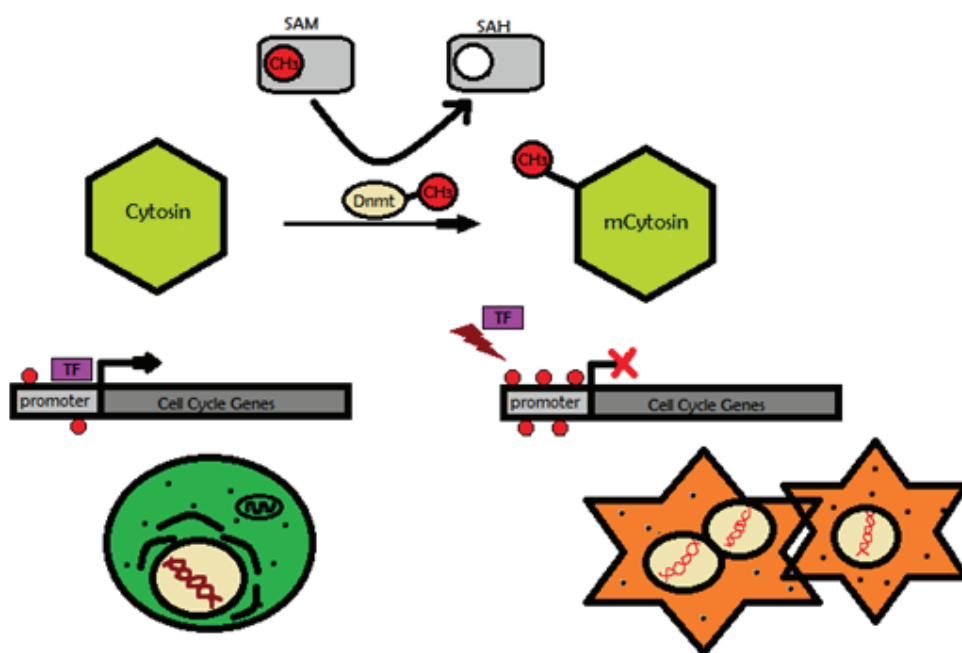
## 5. Histone acetylation

The main action of HDACs is opposite that of histone acetyltransferases. These opposing roles of HATs and HDACs regulate the homeostasis of histone acetylation. HDACs act to remove the acetyl groups from lysine residues in non-histone proteins (67).

There are three classes of histone deacetylases:

- Class I contains histone deacetylase 1, 2, 3 and 8 (in the nucleus)
- Class II contains histone deacetylase 4, 5, 6, 7, 9, and 10 (in the nucleus and cytoplasm)
- Class III contains serotonin (SIRT 1-7)
- Class IV contains Histone deacetylase 11 (HDAC 11 plays the role of both Class I and II) (68).

Classes I, II, and IV possess similar sequences and structures, and they require  $Zn^{2+}$  for enzymatic activity. However, the third family (serotonin) shows no structural similarity to the others, and this enzyme requires  $NAD^+$  (nicotinamide adenine dinucleotide) for catalytic function. Class I are nuclear proteins that regulate histone acetylation and alter chromatin structure (67); however, the actions of all members deeply affect cellular function (69). According to Satoshi *et al.*, loss of HDAC1 and HDAC2 activity in tumor cells

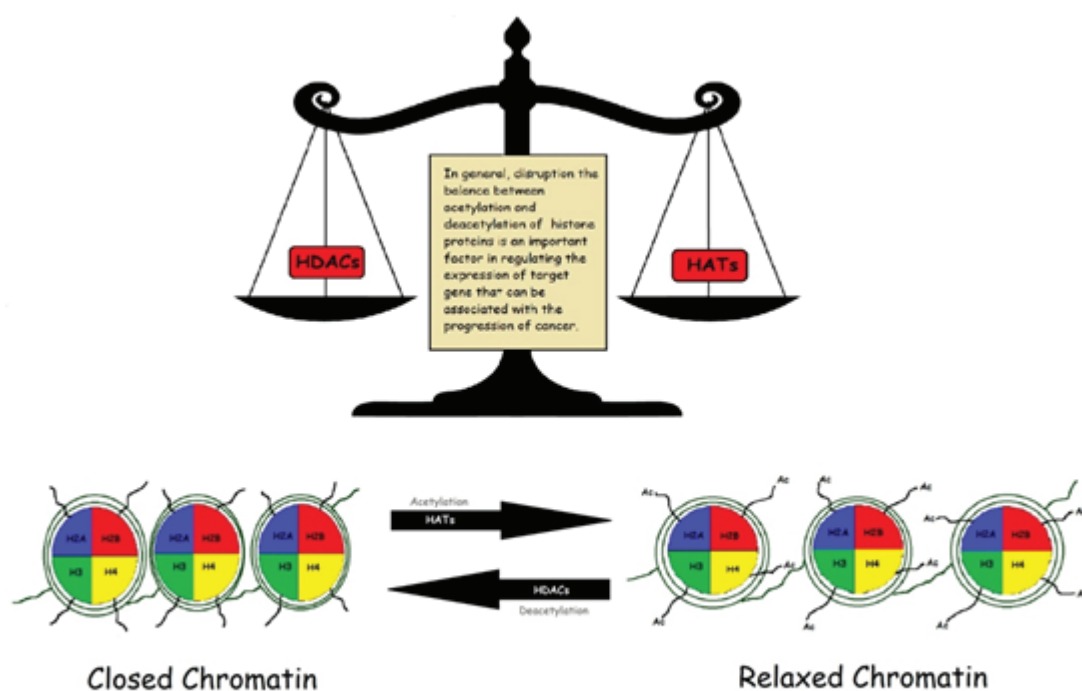


**Figure 2:** DNA methylation. The enzyme DNA methyltransferase (DNMT) catalyzes transfer of a methyl group to the 5C of cytosine to form 5-methylcytosine. S-adenosylmethionine (SAM) is critical for this reaction and acts as a DNA methyltransferase (DNMT) cofactor and a methyl-donor for DNA methylation. During the course of this reaction, SAM is converted to S-adenosyl homocysteine (SAH). DNA methylation changes the affinity of transcription factors to their cognate consensus sequence on the promoter of corresponding genes, ultimately leading to gene expression alterations.

inhibits a particular type of bowel cancer, while loss of HDAC3 has no effect in this context (70). In contrast, it was reported that HDAC3 inactivation can efficiently suppress the growth of intestinal cancer cells (71). Additionally, HDAC3 and HDAC2 inactivation may increase DNA damage and apoptosis (72). Class II and IV histone deacetylases are present in the cytoplasm and usually acetylate non-histone proteins (67). A number of researches have shown the role of HDAC inhibitors in chromatin remodeling and apoptosis (73, 74). There is evidence that alteration in acetylation patterns of non-histone proteins such as HSP90 that are modulated by HDAC6 can affect tumor growth. Conversely, inhibition of HDAC6 activity can stimulate anti-tumor activity (67, 75). Interestingly, deactivation of class II histone deacetylases results in a specific functional outcome. Loss of HDAC4 activity inhibits the proliferation of tumor cells and stimulate apoptosis (76). Additionally, although loss of HDAC7 activity in endothelial cells does not affect cell growth, this loss does inhibit cell migration and results in modification of cell structure (77). Another role of class II of histone deacetylases is to regulate angiogenesis through the function of HDAC6 and HDAC10. Inhibition of HDAC6 and HDAC10 may reduce transcription of vascular epithelial growth factor receptors (VEGFR1 and VEGFR2) (78). Generally, HDAC 1 functions mainly in cellular invasion while HDACII acts in the context of cellular migration, angiogenesis, and cell morphology (79, 80). Changes of the transcriptional level of HDACs within tumor tissues have also been reported. For example, HDAC 1 has a higher level of expression in prostate, stomach, and intestinal tumors



when compared to expression levels in their normal counter parts (71, 81-83). HDAC2 gene expression has been reported in intestinal (84), head and neck (70), and gastric (85) cancers; however, increased levels of HDAC6 expression have been reported in breast cancer (86). Changes in the expression of histone-deacetylase enzymes can alter the level of deacetylation in various genes. For example, methylation of the DAP kinase gene, which encodes an apoptosis regulatory protein, and deacetylation of histones H3 and H4 in the promoter region cause silencing of corresponding genes in tumors of the stomach and the intestine (55, 87).



**Figure 3:** Interplay between histone acetylation and deacetylation. Acetylation of histone tails is catalyzed by histone acetyltransferases (HAT) that relax chromatin structure. In contrast, histone deacetylation mediated by histone deacetylases (HDAC) induces chromatin compaction and gene suppression. Disruption of the balance between acetylation/deacetylation of histone proteins leads to gene expression alterations that may be associated with carcinogenesis.

## 6. Histone Methylation

Lysine residues of histone proteins can be mono, di, or tri-methylated. Binding of a methyl group creates a new level of complexity in the structure of histone protein. Previous studies indicated that these methylation patterns function directly to either activate or inactivate translation (88). Methylation on lysines 4 and 27 in histone H3 has been more widely studied than others. Results showed that it is catalyzed by multisubunit

complexes. The KMT2A (K-specific methyltransferase 2A), also known as MLL, methylates lysine 4 through the action of its regulatory domain (89), and the PRC2 (Polycomb repressive complex) methylates lysine 27 (90). Although the consequences of H3 lysines 4 and 27 methylation in regard to activation or inactivation of gene transcription have not been established, it has been demonstrated that these modifications can restore the chromatin structure of BAF (91). Following methylation of H3 lysine 27, the PRC2 complex detects tri-methyl lysine by a chromodomain containing CBX1, which ultimately serves to increase chromatin compaction and transcription suppression (92-94). As lysine methylation of histone proteins is important for gene expression, the removal of methyl groups is precisely controlled by several lysine demethylase (KDMs) enzymes, including KDM1 (LSD1), KDM6B (JARID1), KDM6A (utx) and KDM6B (JMJD3) (88). The accurate balance between methylation and demethylation of histone proteins is important for precise regulation of gene transcription. Arginine residues are also a target for methylation changes that affect the level of gene expression (95). Arginine methylation acts in two ways to control gene expression. Specifically, methylation of arginine residues that exist in proximity to lysine residues can prevent lysine methylation (96). For example, methylation of arginine 2 inhibits methylation of lysine 3 (97). Methyl arginine can also provide a suitable target for the attachment of methyl-arginine-binding proteins that alter the function of transcriptional regulatory proteins (98).

## 7. Phosphorylation, Ubiquitylation, and Sumoylation

Histone phosphorylation is a dynamic reaction that is targeted to N-terminal serine, threonine, and tyrosine amino acid residues (99). In all creatures from yeast to human, serine 10 of histone H3 (H3S10) is the target amino acid for phosphorylation (100). Hyper phosphorylation of histone H3 has been described in gastric cancer to be associated with invasion, angiogenesis, and metastasis of lymph nodes (101). Levels of histone phosphorylation are regulated by kinase and phosphatase enzymes (102). All known histone kinases catalyze the transfer of phosphate group from ATP to the free OH group of the recipient amino acid. This reaction increases negative charge in the histone protein that affects chromatin structure. The mechanism by which kinase enzymes bind to DNA remains poorly understood (103). Phosphorylation, sumoylation, and ubiquitylation can lead to the activation or inactivation of target genes, and this is dependent up on the site of the reaction. For example, ubiquitylation of H2A lysine 119 is associated with suppression of gene transcription, whereas ubiquitylation of H2B lysine 123 can activate gene transcription (94). Ubiquitylation of H2B lysine 123 participates in gene activation by Ubp8 and Ubp10 proteases (104). Sumoylation, the only histone modification that occurs post-transcription, is defined as an inhibitory mechanism in yeast (105).

## 8. microRNA

A group of 22-nucleotide miRNA fragments can inhibit the expression of target mRNA, can prevent translation, and in some cases function to degrade complementary mRNA.



TABLE 1: Various types of epigenetic alterations that occur during gastric carcinogenesis.

Modification	Frequency	Cellular process	Target genes	References
DNA hypermethylation	Decrease	Signaling pathway	ADAMTS9, BCL68, BNIP3, DAPK, DKK1, FBLN1, GATA4, LMX1A, OPCML, RELN, SFRP protein, SOCS1, SOX17, TIMP3, VEZT, hDAB2IP, RASSF1A, RKIP, SOCS-1, APC, Dkk-3, CRBP1, RAR B, BINP3, PRDM5, TCF4, HAI-2/SPINT2, CXCL12, HOXD10, HOXA1, HoxD10, DLL1, NDRG2, SHP1, CACNA1G, CMTM3, PCDH10, GSTP1, PCDH10, RBP1, SFRP2, GPX3, DAPK, P16	(8, 106-157)
		Transcription regulation	ZNF545, CDH5, HLTF, RUNX3	(79, 80, 108, 126, 143, 145, 158)
		DNA repair	hMLH1, MGMT	(130, 143, 147, 159-162)
		Attachment, invasion and cell migration	CDH1, FLNc, GRIK2, HOXA10, LOX, TIMP3, TSP1	(125, 126, 130, 142, 143, 145, 147, 157, 163-165)
		Chromatin-modifying enzyme	(DNMT1, DNMT3A, DNMT3B, UHRF1)	(166, 167)
		microRNA coding	Let-7f, MIR10B, MIR34C, MIR137, MIR155, MIR182, MIR195, MIR200B, MIR200C, MIR210, MIR212, MIR338, MIR375, MIR378, MIR429, MIR449	(168-179)
DNA hypomethylation	Increase	Signaling pathway	ALDH2, ASCL2, MTHFR, SULF1, SULF2, TERF2, CDKN1C	(126, 180-184)
			MicroRNA gene (MIR93)	-185
H3/H4 Hyperacetylation	Increase	Cell cycle control	MYC	-113
H3/H4 deacetylation	Decrease	Chromatin-modifying enzyme	GATA, RND3	(186, 187)
H3 dephosphorylation	Decrease	Cell cycle control	c-JUN, HSP70	-188
Micro RNAs	Decrease	DNA repair	MGMT, SMARCA5	(189, 190)

Evidence suggests that the regulation of miRNA expression occurs through inappropriate methylation of the regulatory region. For example, methylation of the miR-137 promoter reduces the expression of tumor suppressor genes in stomach cancer, and this has also been reported for other miRNAs such as miR-335, miR-495, miR-9, miR-10b, miR-219-2-3P, miR-212, miR-941, and miR-1247 (191). There are also micro-RNAs that possess dual functions in gastric cancer, and these can act as oncogenes (miR-19a) and tumor suppressors (miR-874) (192).

## 9. Epigenetics Provides a New Approach for Cancer Treatment

Various epimutations such as abnormal methylation have been observed in various cancer cells. These epimutations can be considered as biomarkers for the classification of tumors. One of the most important epimutation alterations is hypermethylation that suppresses the expression of tumor suppressor genes, a process that can ultimately result in tumorigenesis (7, 8, 193). Scientists believe that DNA methyltransferase inhibitors can effectively return cells to normal conditions. These inhibitors are considered as potential drug candidates, as some of them have shown promise in *in vitro* pharmacogenetic analyses in mice (193, 194, 195). Similar to methyltransferase inhibitors, histone deacetylase inhibitors may also prove effective in the epigenetic treatment of cancer. Collectively, there are two categories of epigenetic drugs:

1. DNA methylation inhibitors:

- (a) 5-azacitidine (vidaza)
- (b) Decitabine (2-deoxy-5-azacitidine)

2. Histone deacetylase inhibitors:

- (a) Suberanilohydroxamic acid (SAHA, Zolina)
- (b) Romidepsin (Istodox) (196)

Azacididine and Decitabine have both proven to be beneficial in the treatment of myelodysplastic syndrome (197, 198) and myeloid leukemia. Both medication strategies are effective when ordered at low doses (199). Over the past few years, various drugs have been discovered for cancer treatment; however, most of these lack specificity. Non-specific effects of anticancer drugs are likely due to their mechanism of action or their wide range of target substrates. Given the significant role of epigenetic modifications in the context of cancer incidence, anticancer drugs should function to preserve the normal epigenetic state.

## 10. Conclusions and Future Direction

The epigenomic profile of a cell is dependent upon the status of DNA methylation, histone proteins modifications, and non-coding RNAs working individually or in a network to support either transcriptional activation or suppression of genes. In diseases such as cancer, aberrant epigenetic modifications may activate or suppress transcription of oncogenes or tumor suppressor genes, respectively. The main advantage of epigenetic therapies is that, despite genetic abnormalities, epigenetic alterations are reversible. The goal of epigenetic therapies is to reverse neoplastic growth of tumor cells to a more normal state. Additionally, epigenetic differences between individuals provide opportunities to create personalized medications.

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## Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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