Research in Molecular Medicine (RMM)
Volume 6, Issue no. 3, DOI 10.18502/rmm.v6i3.4606
Production and Hosting by Knowledge E

Review Article

An Overview of the Epigenetic Modifications of Gene Expression in Tumorigenesis

Leili Sadeghi Amiri[1], Ali Barzegar[1], Alireza Rafiei[2], and Omolbanin Amjadi[3]

[1]Department of Basic Sciences, Sari Agriculture Science and Natural Resources University, Sari, Iran
[2]Department of Immunology, Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[3]Department of Molecular genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

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.

Keywords: Epigenetic, Gastric cancer, Gene expression, Methylation.

Corresponding Author:
Ali Barzegar;
email: alibar647@yahoo.com

Production and Hosting by Knowledge E

Editor-in-Chief:
Dr. Alireza Rafiei

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

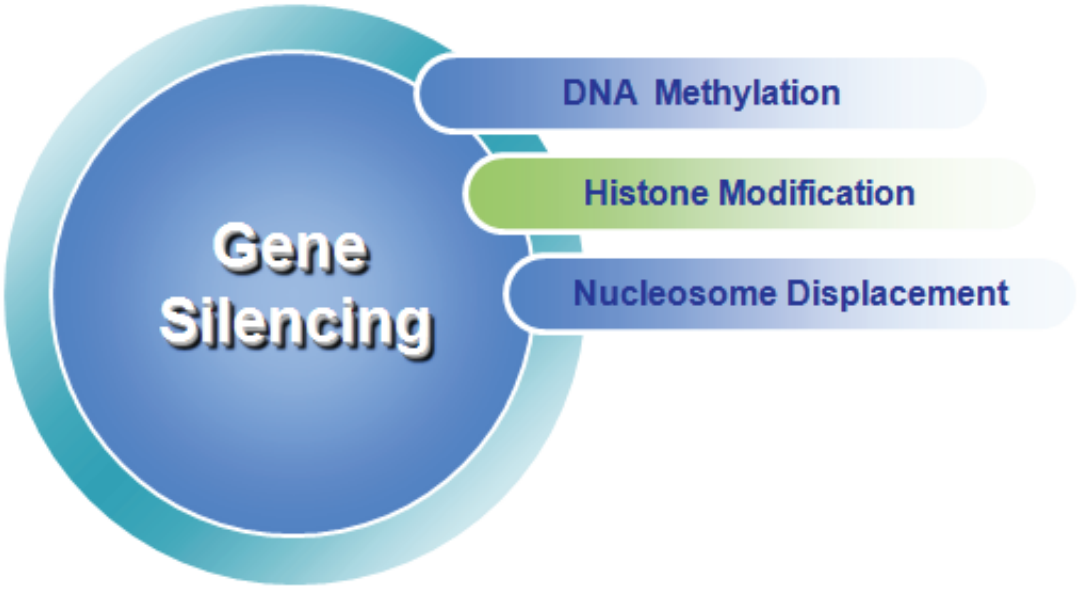

Figure 1: Hereditary of gene silencing is regulated by mechanisms that include DNA methylation, histone modification, and nucleosome displacement. DNA methyltransferase (DNMT), histone deacetylase (HDAC), histone methyltransferases (HMTs), and nucleosomal alteration factors (NURFs) are involved in DNA 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 indifferent 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 translational 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 histoneoctamer 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 homeostas 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 / Myth
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 alleleis correlated with malignancies such as lymphoma, ovarian, and head and neck tumors (64).

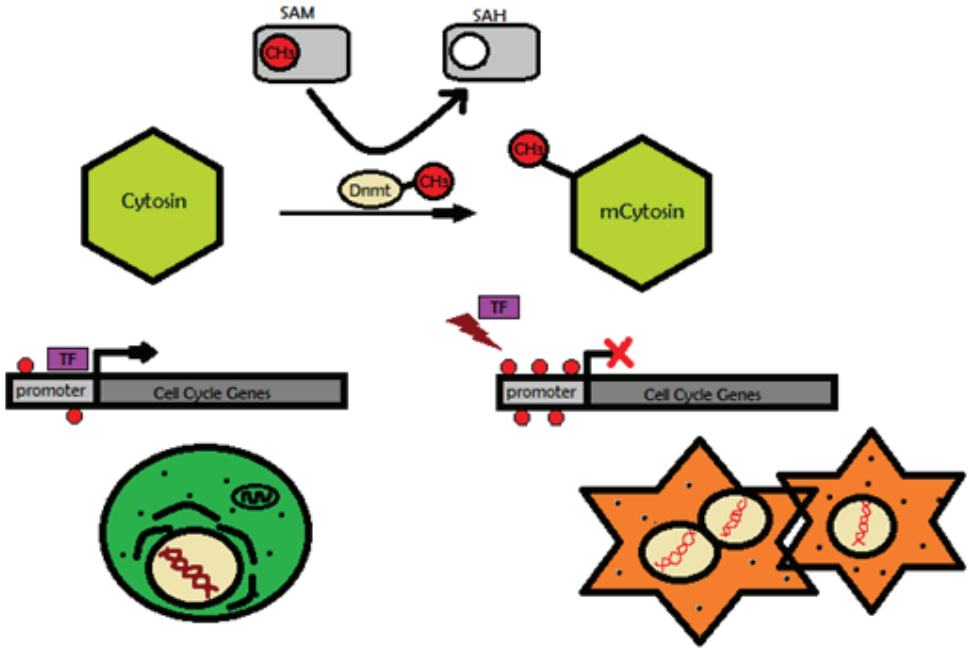

Figure 2: DNA methylation. The enzyme DNA methyltransferase (DNMT) catalyzes transfer of a methyl group to the 5C of cytosine to form 5-methylcytosine. S-adenosylmethionine (SAM) is critical for this reaction and acts as a DNA methyltransferase (DNMT) cofactor and a methyl-donor for DNA methylation. 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.

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 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 sapoptosis (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).

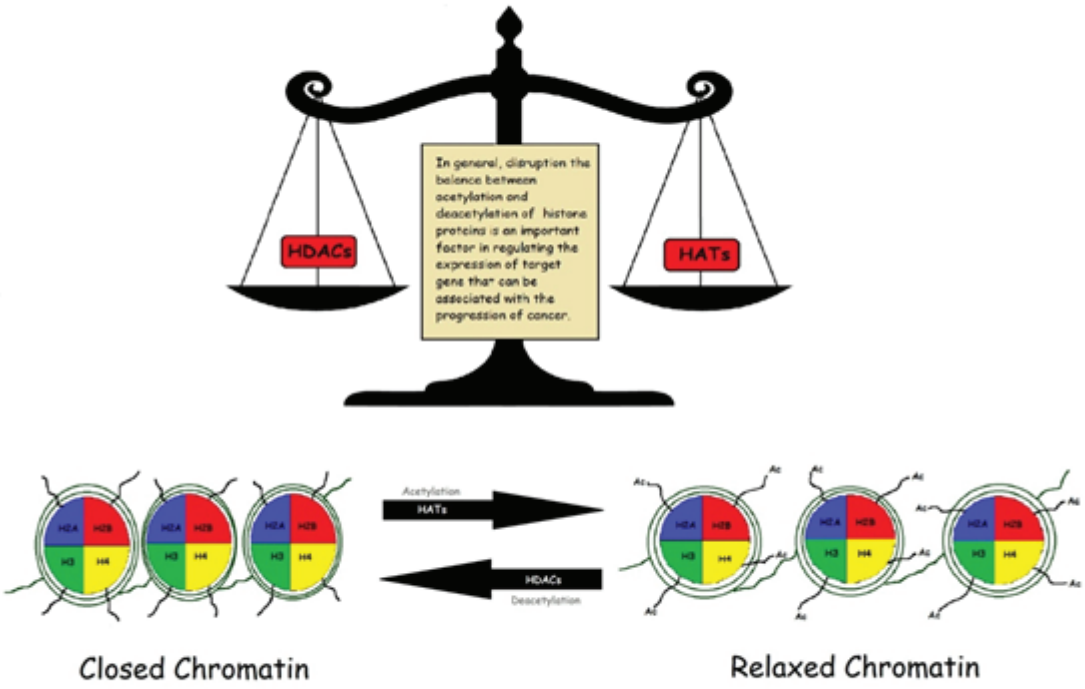

Figure 3: Interplay between histone acetylation and deacetylation. Acetylation of histone tails is catalyzed by histone acetyltransferases (HAT) that relax chromatin structure. In contrast, histone deacetylation mediated by histone deacetylases (HDAC) induces chromatin compaction and gene suppression. 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-pecific 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 methylate-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 mechanismin 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. 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:

Table 1: Various types of epigenetic alterations that occurduring 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, *h*DAB2IP, 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)
	Increase		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)

(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.

Acknowledgements

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

[1] Toutounchi M, Shahhosseini M, Moumeni Moghadam M, Baharvand H. Epigenetic of stem cells. Yakhteh. 2008; 9(1): 51–66.

[2] Tronick E, Hunter RG. Waddington, dynamic systems, epigenetics. Frontiers in Behavioral Neuroscience. 2016; 10:107.

[3] Ghaedi K, Miri M, Tavassoli M. Process of epigenetic in cancer. Genetic in 3rd Millennium. 2007; 5(4): 1191-1195.

[4] Bird A. Perceptions of epigenetics. Nature. 2007; 447(7143): 396-8.

[5] Feinberg AP, Tycko B. The history of cancer epigenetics. Nature Reviews Cancer. 2004; 4(2): 143-53.

[6] Hinshelwood RA, Clark SJ. Breast cancer epigenetics: normal human mammary epithelial cells as a model system. Journal of Molecular Medicine. 2008; 86(12): 1315-28.

[7] Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nature Reviews Genetics. 2007; 8(4): 286-98.

[8] Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007; 128(4): 683-92.

[9] Pfeifer GP, Besaratinia A. Mutational spectra of human cancer. Human Genetics. 2009; 125(5-6): 493-506.

[10] Bird A. DNA methylation patterns and epigenetic memory. Genes and Development. 2002; 16(1): 6-21.

[11] Stearns V, Zhou Q, Davidson NE. Epigenetic regulation as a new target for breast cancer therapy. Cancer Investigation. 2007; 25(8): 659-65.

[12] Abbasi B, Ansari Nejad N, Fardad F, Nasiripour S, Ramim T. Breast cancer epigenetics: review article. Tehran University Medical Journal TUMS Publications. 2016; 74(8): 535-44.

[13] Frigola J, Song J, Stirzaker C, Hinshelwood RA, Peinado MA, Clark SJ. Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. Nature Genetics. 2006; 38(5): 540-9.

[14] Byun DS, Lee MG, Chae KS, Ryu BG, Chi SG. Frequent epigenetic inactivation of RASSF1A by aberrant promoter hypermethylation in human gastric adenocarcinoma. Cancer Research. 2001; 61(19): 7034-8.

[15] Nojima M, Suzuki H, Toyota M, Watanabe Y, Maruyama R, Sasaki S, et al. Frequent epigenetic inactivation of SFRP genes and constitutive activation of Wnt signaling in gastric cancer. Oncogene. 2007; 26(32): 4699-713.

[16] Zeng XQ, Wang J, Chen SY. Methylation modification in gastric cancer and approaches to targeted epigenetic therapy. International Journal of Oncology. 2017; 50(6): 1921-33.

[17] Choi SJ, Jung SW, Huh S, Chung YS, Cho H, Kang H. Alteration of DNA Methylation in Gastric Cancer with Chemotherapy. Journal of Microbiology and Biotechnology. 2017; 27(8): 1367-78.

[18] Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007; 128(4): 669-81.

[19] Lande-Diner L, Cedar H. Silence of the genes—mechanisms of long-term repression. Nature Reviews Genetics. 2005; 6(8): 648-54.

[20] Lorincz MC, Dickerson DR, Schmitt M, Groudine M. Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nature Structural and Molecular Biology. 2004; 11(11): 1068-75.

[21] khalaj M, Abarzegar, MA Ebrahimzadeh, A farhadi. Study of the association of cyp3A4 gene promoter methylation with breast cancer incidence in Mazandaran province. The Conference of Novel Findings in Bioscience. 2017.

[22] Dehdari H, A barzegar, F moradian, MA Ebrahimzadeh. Methylation profile of the 5' regulatory region of cyp1A1 gene in breast cancer patients. 3rd National Conference of New Cellular and Molecular,Ardebil. 2017.

[23] Esteller M. Epigenetics in cancer. New England Journal of Medicine. 2008; 358(11): 1148-59.

[24] Tahara E. Molecular mechanism of stomach carcinogenesis. Journal of Cancer Research and Clinical Oncology. 1993; 119(5): 265-72.

[25] Delgado S, Gómez M, Bird A, Antequera F. Initiation of DNA replication at CpG islands in mammalian chromosomes. The EMBO Journal. 1998; 17(8): 2426-35.

[26] Shim YH, Kang GH, Ro JY. Correlation of p16 hypermethylation with p16 protein loss in sporadic gastric carcinomas. Laboratory Investigation. 2000; 80(5): 689-95.

[27] Leung SY, Yuen ST, Chung LP, Chu KM, Chan AS, Ho JC. hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. Cancer Research. 1999; 59(1): 159-64.

[28] Kang SH, Choi HH, Kim SG, Jong HS, Kim NK, Kim SJ, et al. Transcriptional inactivation of the tissue inhibitor of metalloproteinase−3 gene by dna hypermethylation of the 5'−CpG island in human gastric cancer cell lines. International Journal of Cancer. 2000; 86(5): 632-5.

[29] Bevilacqua RA, Simpson AJ. Methylation of the hMLH1 promoter but no hMLH1 mutations in sporadic gastric carcinomas with high−level microsatellite instability. International Journal of Cancer. 2000; 87(2): 200-3.

[30] Lee YY, Kang SH, Seo JY, Jung CW, Lee KU, Choe KJ, et al. Alterations of p16INK4A and p15INK4B genes in gastric carcinomas. Cancer. 1997; 80(10): 1889-96.

[31] Kissil JL, Feinstein E, Cohen O, Jones PA, Tsai YC, Knowles MA, et al. DAP-kinase loss of expression in various carcinoma and B-cell lymphoma cell lines: possible implications for role as tumor suppressor gene. Oncogene. 1997; 15(4): 403-7.

[32] Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Research. 1999; 59(1): 67-70.

[33] Katzenellenbogen RA, Baylin SB, Herman JG. Hypermethylation of the DAP-kinase CpG island is a common alteration in B-cell malignancies. Blood. 1999; 93(12): 4347-53.

[34] Roberts DD. Regulation of tumor growth and metastasis by thrombospondin-1. The FASEB Journal. 1996; 10(10): 1183-91.

[35] Li Q, Ahuja N, Burger PC, Issa J-PJ. Methylation and silencing of the Thrombospondin-1 promoter in human cancer. Oncogene. 1999; 18(21): 3284-9.

[36] Yang N, Zhang L, Zhang Y, Kazazian Jr HH. An important role for RUNX3 in human L1 transcription and retrotransposition. Nucleic Acids Research. 2003; 31(16): 4929-40.

[37] Kouraklis G, Theocharis S. Histone deacetylase inhibitors and anticancer therapy. Current Medicinal Chemistry-Anti-Cancer Agents. 2002; 2(4): 477-84.

[38] Schübeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, Van Leeuwen F, et al. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes & Development. 2004; 18(11): 1263-71.

[39] Xu D, Bai J, Duan Q, Costa M, Dai W. Covalent modifications of histones during mitosis and meiosis. Cell Cycle. 2009; 8(22): 3688-94.

[40] Mellor J. The dynamics of chromatin remodeling at promoters. Molecular Cell. 2005; 19(2): 147-57.

[41] Altieri F, Di Stadio CS, Federico A, Miselli G, De Palma M, Rippa E, et al. Epigenetic alterations of gastrokine 1 gene expression in gastric cancer. Oncotarget. 2017; 8(10): 16899.

[42] Nan X, Ng H-H, Johnson CA, Laherty CD, Turner BM, Eisenman RN, et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature. 1998; 393(6683): 386-9.

[43] Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. Nature Genetics. 2000; 25(3): 338-42.

[44] Bachman KE, Rountree MR, Baylin SB. Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. Journal of Biological Chemistry. 2001; 276(34): 32282-7.

[45] Rose NR, Klose RJ. Understanding the relationship between DNA methylation and histone lysine methylation. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2014; 1839(12): 1362-72.

[46] Rea S, Eisenhaber F, O'carroll D, Strahl BD, Sun Z-W, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature. 2000; 406(6796): 593-9.

[47] De Murcia G, Shall S. From DNA damage and stress signalling to cell death: poly ADP-ribosylation reactions: Oxford University Press on Demand; 2000.

[48] Zardo G, Reale A, Matteis GD, Buontempo S, Caiafa P. A role for poly (ADP-ribosyl) ation in DNA methylation. Biochemistry and Cell Biology. 2003; 81(3): 197-208.

[49] Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nature Genetics. 2005; 37(4): 391-400.

[50] Seligson DB, Horvath S, Shi T, Yu H. Global histone modification patterns predict risk of prostate cancer recurrence. Nature. 2005; 435(7046): 1262.

[51] Allfrey V, Faulkner R, Mirsky A. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proceedings of the National Academy of Sciences. 1964; 51(5): 786-94.

[52] Zhou S TA, Lupien M. Emergence of the noncoding cancer genome: a target of genetic and epigenetic alterations. Cancer Discovery. 2016 Nov; 6(11): 1215-29.

[53] Forsberg EC, Bresnick EH. Histone acetylation beyond promoters: long–range acetylation patterns in the chromatin world. Bioessays. 2001; 23(9): 820-30.

[54] Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. Nature Reviews Drug Discovery. 2002; 1(4): 287-99.

[55] Fraga MF BE, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG. Histone deacetylases and cancer: causes and therapies. Nature Reviews Cancer. 2001; 1(3): 194-202.

[56] Timmermann S, Lehrmann H, Polesskaya A, Harel-Bellan A. Histone acetylation and disease. Cellular and Molecular Life Sciences. 2001; 58(5): 728-36.

[57] Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997; 389(6649): 349.

[58] Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, Part I: Covalent histone modifications. Trends in Molecular Medicine. 2007; 13(9): 363-72.

[59] Inche AG, La Thangue NB. Keynote review: Chromatin control and cancer-drug discovery: realizing the promise. Drug Discovery Today. 2006; 11(3): 97-109.

[60] Yasui W, Oue N, Ono S, Mitani Y, Ito R, Nakayama H. Histone acetylation and gastrointestinal carcinogenesis. Annals of the New York Academy of Sciences. 2003; 983(1): 220-31.

[61] Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. Gene. 2005; 363: 15-23.

[62] Davis PK, Brachmann RK. Chromatin remodeling and cancer. Cancer Biology and Therapy. 2003; 2(1): 23-30.

[63] Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin S-F, et al. Mutations truncating the EP300 acetylase in human cancers. Nature Genetics. 2000; 24(3): 300.

[64] Gorrini C, Squatrito M, Luise C, Syed N, Perna D, Wark L, et al. Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. Nature. 2007; 448(7157): 1063.

[65] Sykes SM, Mellert HS, Holbert MA, Li K, Marmorstein R, Lane WS, et al. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. Molecular Cell. 2006; 24(6): 841-51.

[66] Avvakumov N, Cote J. The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene. 2007; 26(37): 5395.

[67] Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nature Reviews Drug Discovery. 2006; 5(9): 769.

[68] Glozak M, Seto E. Histone deacetylases and cancer. Oncogene. 2007;26(37):5420.

[69] Witt O, Deubzer HE, Milde T, Oehme I. HDAC family: What are the cancer relevant targets?. Cancer Letters. 2009; 277(1): 8-21.

[70] Inoue S, Mai A, Dyer MJ, Cohen GM. Inhibition of histone deacetylase class I but not class II is critical for the sensitization of leukemic cells to tumor necrosis factor–related apoptosis-inducing ligand–induced apoptosis. Cancer Research. 2006; 66(13): 6785-92.

[71] Wilson AJ, Byun D-S, Popova N, Murray LB, L'Italien K, Sowa Y, et al. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. Journal of Biological Chemistry. 2006; 281(19): 13548-58.

[72] Nakagawa M, Oda Y, Eguchi T, Aishima S-I, Yao T, Hosoi F, et al. Expression profile of class I histone deacetylases in human cancer tissues. Oncology Reports. 2007;18(4):769-74.

[73] Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, et al. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(2): 540-5.

[74] Peart MJ, Smyth GK, van Laar RK, Bowtell DD, Richon VM, Marks PA, et al. Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(10): 3697-702.

[75] Haggarty SJ KK, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proceedings of the National Academy of Sciences. 2003; 100(8): 4389-94.

[76] Wilson AJ, Byun D-S, Nasser S, Murray LB, Ayyanar K, Arango D, et al. HDAC4 promotes growth of colon cancer cells via repression of p21. Molecular Biology of the Cell. 2008; 19(10): 4062-75.

[77] Mottet D, Bellahcène A, Pirotte S, Waltregny D, Deroanne C, Lamour V, et al. Histone deacetylase 7 silencing alters endothelial cell migration, a key step in angiogenesis. Circulation Research. 2007; 101(12): 1237-46.

[78] Park JH, Kim SH, Choi MC, Lee J, Oh DY, Im SA, et al. Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors. Biochemical and Biophysical Research Communications. 2008; 368(2): 318-22.

[79] Wang S, Cheng Y, Du W, Lu L, Zhou L, Wang H, et al. Zinc-finger protein 545 is a novel tumour suppressor that acts by inhibiting ribosomal RNA transcription in gastric cancer. Gut. 2013; 62(6): 833-841

[80] Wang X, Lau KK, So LK, Lam YW. CHD5 is down-regulated through promoter hypermethylation in gastric cancer. Journal of Biomedical Science. 2009; 16(1): 95.

[81] Choi JH, Kwon HJ, Yoon BI, Kim JH, Han SU, Joo HJ, et al. Expression profile of histone deacetylase 1 in gastric cancer tissues. Cancer Science. 2001; 92(12): 1300-4.

[82] Halkidou K, Gaughan L, Cook S, Leung HY, Neal DE, Robson CN. Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. The Prostate. 2004; 59(2): 177-89.

[83] Zhang Z, Yamashita H, Toyama T, Sugiura H, Ando Y, Mita K, et al. Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast. Breast Cancer Research and Treatment. 2005;94(1): 11-6.

[84] Zhu P, Martin E, Mengwasser J, Schlag P, Janssen K-P, Göttlicher M. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. Cancer Cell. 2004; 5(5): 455-63.

[85] Song J, Noh JH, Lee JH, Eun JW, Ahn YM, Kim SY, et al. Increased expression of histone deacetylase 2 is found in human gastric cancer. Apmis. 2005; 113(4): 264-8.

[86] Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y, et al. HDAC6 expression is correlated with better survival in breast cancer. Clinical Cancer Research. 2004; 10(20): 6962-8.

[87] Satoh A, Toyota M, Itoh F, Kikuchi T, Obata T, Sasaki Y, et al. DNA methylation and histone deacetylation associated with silencing DAP kinase gene expression in colorectal and gastric cancers. British Journal of Cancer. 2002; 86(11): 1817-23.

[88] Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. Molecular Cell. 2012;48(4):491-507.

[89] Yokoyama A, Wang Z, Wysocka J, Sanyal M, Aufiero DJ, Kitabayashi I, et al. Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate Hox gene expression. Molecular and Cellular Biology. 2004; 24(13): 5639-49.

[90] Cao R, Zhang Y. The functions of E (Z)/EZH2-mediated methylation of lysine 27 in histone H3. Current Opinion in Genetics and Development. 2004; 14(2): 155-64.

[91] Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, et al. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature. 2006; 442(7098): 86.

[92] Francis NJ, Kingston RE, Woodcock CL. Chromatin compaction by a polycomb group protein complex. Science. 2004; 306(5701): 1574-7.

[93] Akiyama Y, Maesawa C, Ogasawara S, Terashima M, Masuda T. Cell-type-specific repression of the maspin gene is disrupted frequently by demethylation at the promoter region in gastric intestinal metaplasia and cancer cells. The American Journal of Pathology. 2003; 163(5): 1911-9.

[94] Kaustov L, Ouyang H, Amaya M, Lemak A, Nady N, Duan S, et al. Recognition and specificity determinants of the human cbx chromodomains. Journal of Biological Chemistry. 2011; 286(1): 521-9.

[95] Di Lorenzo A, Bedford MT. Histone arginine methylation. FEBS Letters. 2011; 585(13): 2024-31.

[96] Hyllus D, Stein C, Schnabel K, Schiltz E, Imhof A, Dou Y, et al. PRMT6-mediated methylation of R2 in histone H3 antagonizes H3 K4 trimethylation. Genes & Development. 2007; 21(24): 3369-80.

[97] Migliori V, Müller J, Phalke S, Low D, Bezzi M, Mok WC, et al. Symmetric dimethylation of H3R2 is a newly identified histone mark that supports euchromatin maintenance. Nature Structural and Molecular Biology. 2012; 19(2): 136-44.

[98] Liu K, Guo Y, Liu H, Bian C, Lam R, Liu Y, et al. Crystal structure of TDRD3 and methyl-arginine binding characterization of TDRD3, SMN and SPF30. PloS One. 2012; 7(2): e30375.

[99] Xhemalce B DM, Bannister AJ. Histone modifications. Reviews in Cell Biology and Molecular Medicine. 2011.

[100] Kouzarides T BS. Chromatin modifications and their mechanism of action. Epigenetics. 2007; 191: 209.

[101] Takahashi H MY, Tsuneyama K, Nomoto K, Okada E, Fujita H, Takano Y. Over expression of phosphorylated histone H3 is an indicator of poor prognosis in gastric adenocarcinoma patients. Applied Immunohistochemistry & Molecular Morphology. 2006; 14(3): 296-302.

[102] Oki M AH, Ito T Role of histone phosphorylation in chromatin dynamics and its implications in diseases. Chromatin and Disease. 2007: 323-40.

[103] Hu S XZ, Onishi A, Yu X, Jiang L, Lin J, Rho HS, Woodard C, Wang H, Jeong JS, Long S. Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. Cell. 2009; 139(3): 310-622.

[104] Henry KW WA, Lo WS, Duggan LJ, Emre NT, Kao CF, Pillus L, Shilatifard A, Osley MA, Berger SL. . Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes and Development. 2003; 17(21): 2648-63.

[105] Shiio Y ER. Histone sumoylation is associated with transcriptional repression. Proceedings of the National Academy of Sciences. 2003; 100(23): 13225-30.

[106] Cheng Y, Jin H, Liu X, Siu J, Wong Y, Ng E, et al. Fibulin 1 is downregulated through promoter hypermethylation in gastric cancer. British Journal of Cancer. 2008; 99(12): 2083.

[107] Shin CM, Kim N, Jung Y, Park JH, Kang GH, Park WY, et al. Genome–Wide DNA Methylation Profiles in Noncancerous Gastric Mucosae with Regard to *Helicobacter-pylori* Infection and the Presence of Gastric Cancer. Helicobacter. 2011; 16(3): 179-88.

[108] Murai M, Toyota M, Suzuki H, Satoh A, Sasaki Y, Akino K, et al. Aberrant methylation and silencing of the BNIP3 gene in colorectal and gastric cancer. Clinical Cancer Research. 2005; 11(3): 1021-7.

[109] Mikata R, Fukai K, Imazeki F, Arai M, Fujiwara K, Yonemitsu Y, et al. BCL2L10 is frequently silenced by promoter hypermethylation in gastric cancer. Oncology Reports. 2010; 23(6): 1701-8.

[110] Xu JD, Cao XX, Long ZW, Liu XP, Furuya T, Xu JW, et al. BCL2L10 protein regulates apoptosis/proliferation through differential pathways in gastric cancer cells. The Journal of Pathology. 2011; 223(3): 400-9.

[111] Li X, Cheung K, Ma X, Tian L, Zhao J, Go M, et al. Epigenetic inactivation of paired box gene 5, a novel tumor suppressor gene, through direct upregulation of p53 is associated with prognosis in gastric cancer patients. Oncogene. 2012; 31(29): 3419.

[112] Dong W, Feng L, Xie Y, Zhang H, Wu Y. Hypermethylation–mediated reduction of LMX1A expression in gastric cancer. Cancer Science. 2011; 102(2): 361-6.

[113] Akiyama Y, Watkins N, Suzuki H, Jair K-W, van Engeland M, Esteller M, et al. GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. Molecular and Cellular Biology. 2003; 23(23): 8429-39.

[114] Balgkouranidou I, Karayiannakis A, Matthaios D, Bolanaki H, Tripsianis G, Tentes AA, et al. Assessment of SOX17 DNA methylation in cell free DNA from patients with operable gastric cancer. Association with prognostic variables and survival. Clinical Chemistry and Laboratory Medicine. 2013; 51(7): 1505-10.

[115] Bian Y, Wang L, Lu H, Yang G, Zhang Z, Fu H, et al. Downregulation of tumor suppressor QKI in gastric cancer and its implication in cancer prognosis. Biochemical and Biophysical Research Communications. 2012; 422(1): 187-93.

[116] Guo X, Jing C, Li L, Zhang L, Shi Y, Wang J, et al. Down-regulation of VEZT gene expression in human gastric cancer involves promoter methylation and miR-43c. Biochemical and Biophysical Research Communications. 2011; 404(2): 622-7.

[117] Dohi O, Takada H, Wakabayashi N, Yasui K, Sakakura C, Mitsufuji S, et al. Epigenetic silencing of RELN in gastric cancer. International Journal of Oncology. 2010;36(1):85-92.

[118] Guan Z, Zhang J, Song S, Dai D. Promoter methylation and expression of TIMP3 gene in gastric cancer. Diagnostic Pathology. 2013; 8(1): 110.

[119] Du W, Wang S, Zhou Q, Li X, Chu J, Chang Z, et al. ADAMTS9 is a functional tumor suppressor through inhibiting AKT/mTOR pathway and associated with poor survival in gastric cancer. Oncogene. 2013; 32(28): 3319-28.

[120] Piazzi G, Fini L, Selgrad M, Garcia M, Daoud Y, Wex T, et al. Epigenetic regulation of Delta-Like1 controls Notch1 activation in gastric cancer. Oncotarget. 2011; 2(12): 1291.

[121] Mikata R, Yokosuka O, Fukai K, Imazeki F, Arai M, Tada M, et al. Analysis of genes upregulated by the demethylating agent 5–aza–2′–deoxycytidine in gastric cancer cell lines. International Journal of Cancer. 2006; 119(7): 1616-22.

[122] Suzuki H, Itoh F, Toyota M, Kikuchi T, Kakiuchi H, Hinoda Y, et al. Distinct methylation pattern and microsatellite instability in sporadic gastric cancer. International Journal of Cancer. 1999; 83(3): 309-13.

[123] Zhi Y, Chen J, Zhang S, Chang X, Ma J, Dai D. Down-regulation of CXCL12 by DNA hypermethylation and its involvement in gastric cancer metastatic progression. Digestive Diseases and Sciences. 2012; 57(3): 650-9.

[124] Leung WK, To KF, Chu ES, Chan MW, Bai AH, Ng EK, Chan FK, Sung JJ. Potential diagnostic and prognostic values of detecting promoter hypermethylation in the serum of patients with gastric cancer. British Journal of Cancer. 2005; 92(12): 2190.

[125] Tahara T, Shibata T, Nakamura M, Yamashita H, Yoshioka D, Okubo M, et al. Increased number of CpG island hypermethylation in tumor suppressor genes of non-neoplastic gastric mucosa correlates with higher risk of gastric cancer. Digestion. 2010; 82(1): 27-36.

[126] Kang GH, Lee S, Cho N-Y, Gandamihardja T, Long TI, Weisenberger DJ, et al. DNA methylation profiles of gastric carcinoma characterized by quantitative DNA methylation analysis. Laboratory investigation. 2008; 88(2): 161.

[127] Dote H, Toyooka S, Tsukuda K, Yano M, Ota T, Murakami M, et al. Aberrant promoter methylation in human DAB2 interactive protein (hDAB2IP) gene in gastrointestinal tumour. British Journal of Cancer. 2005; 92(6): 1117.

[128] Yao D, Shi J, Shi B, Wang N, Liu W, Zhang G, et al. Quantitative assessment of gene methylation and their impact on clinical outcome in gastric cancer. Clinica Chimica Acta. 2012; 413(7): 787-94.

[129] Agathanggelou A, Cooper WN, Latif F. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. Cancer Research. 2005; 65(9): 3497-508.

[130] Ksiaa F, Ziadi S, Amara K, Korbi S, Trimeche M. Biological significance of promoter hypermethylation of tumor-related genes in patients with gastric carcinoma. Clinica Chimica Acta. 2009; 404(2): 128-33.

[131] Guo W, Dong Z, Guo Y, Lin X, Chen Z, Kuang G, et al. Aberrant methylation and loss expression of RKIP is associated with tumor progression and poor prognosis in gastric cardia adenocarcinoma. Clinical & Experimental Metastasis. 2013; 30(3): 265-75.

[132] To K, Chan M, Leung W, Ng E, Yu J, Bai A, et al. Constitutional activation of IL-6-mediated JAK/STAT pathway through hypermethylation of SOCS-1 in human gastric cancer cell line. British Journal of Cancer. 2004; 91(7): 1335.

[133] Souma Y, Nishida T, Serada S, Iwahori K, Takahashi T, Fujimoto M, et al. Antiproliferative effect of SOCS–1 through the suppression of STAT3 and p38 MAPK activation in gastric cancer cells. International Journal of Cancer. 2012;131(6):1287-96.

[134] Radulescu S, Ridgway R, Cordero J, Athineos D, Salgueiro P, Poulsom R, et al. Acute WNT signalling activation perturbs differentiation within the adult stomach and rapidly leads to tumour formation. Oncogene. 2013; 32(16): 2048.

[135] Deng G, Song G-A, Pong E, Sleisenger M, Kim YS. Promoter methylation inhibits APC gene expression by causing changes in chromatin conformation and interfering with the binding of transcription factor CCAAT-binding factor. Cancer Research. 2004; 64(8): 2692-8.

[136] Yu J, Tao Q, Cheng YY, Lee KY, Ng SS, Cheung KF, et al. Promoter methylation of the Wnt/β–catenin signaling antagonist Dkk–3 is associated with poor survival in gastric cancer. Cancer. 2009; 115(1): 49-60.

[137] Guo Y, Guo W, Chen Z, Kuang G, Yang Z, Dong Z. Hypermethylation and aberrant expression of Wnt-antagonist family genes in gastric cardia adenocarcinoma. Neoplasma. 2011; 58(2): 110-7.

[138] Kobayashi K, Ouchida M, Tsuji T, Hanafusa H, Miyazaki M, Namba M, et al. Reduced expression of the REIC/Dkk-3 gene by promoter-hypermethylation in human tumor cells. Gene. 2002; 282(1): 151-8.

[139] Shutoh M, Oue N, Aung PP, Noguchi T, Kuraoka K, Nakayama H, et al. DNA methylation of genes linked with retinoid signaling in gastric carcinoma. Cancer. 2005; 104(8): 1609-19.

[140] Jeronimo C, Henrique R, Oliveira J, Lobo F, Pais I, Teixeira M, et al. Aberrant cellular retinol binding protein 1 (CRBP1) gene expression and promoter methylation in prostate cancer. Journal of Clinical Pathology. 2004; 57(8): 872-6.

[141] Narayan G, Arias-Pulido H, Koul S, Vargas H, Zhang FF, Villella J, et al. Frequent promoter methylation of CDH1, DAPK, RARB, and HIC1 genes in carcinoma of cervix uteri: its relationship to clinical outcome. Molecular Cancer. 2003; 2(1): 24.

[142] Ben Ayed–Guerfali D, Benhaj K, Khabir A, Abid M, Bayrouti MI, Sellami–Boudawara T, et al. Hypermethylation of tumor–related genes in tunisian patients with gastric carcinoma: Clinical and biological significance. Journal of Surgical Oncology. 2011; 103(7): 687-94.

[143] Oue N, Mitani Y, Motoshita J, Matsumura S, Yoshida K, Kuniyasu H, et al. Accumulation of DNA methylation is associated with tumor stage in gastric cancer. Cancer. 2006; 106(6): 1250-9.

[144] Yamashita S, Tsujino Y, Moriguchi K, Tatematsu M, Ushijima T. Chemical genomic screening for methylation–silenced genes in gastric cancer cell lines using 5–aza–2′–deoxycytidine treatment and oligonucleotide microarray. Cancer Science. 2006; 97(1): 64-71.

[145] Hiraki M, Kitajima Y, Koga Y, Tanaka T, Nakamura J, Hashiguchi K, et al. Aberrant gene methylation is a biomarker for the detection of cancer cells in peritoneal wash samples from advanced gastric cancer patients. Annals of Surgical Oncology. 2011; 18(10): 3013-9.

[146] Sugita H, Iida S, Inokuchi M, Kato K, Ishiguro M, Ishikawa T, et al. Methylation of BNIP3 and DAPK indicates lower response to chemotherapy and poor prognosis in gastric cancer. Oncology Reports. 2011; 25(2): 513-8.

[147] Shi J, Zhang G, Yao D, Liu W, Wang N, Ji M, et al. Prognostic significance of aberrant gene methylation in gastric cancer. American Journal of Cancer Research. 2012; 2(1): 116.

[148] Joo JK, Kim SH, Kim HG, Kim DY, Ryu SY, Lee KH, et al. CpG methylation of transcription factor 4 in gastric carcinoma. Annals of Surgical Oncology. 2010; 17(12): 3344-53.

[149] Shu X-s, Geng H, Li L, Ying J, Ma C, Wang Y, et al. The epigenetic modifier PRDM5 functions as a tumor suppressor through modulating WNT/β-catenin signaling and is frequently silenced in multiple tumors. PloS One. 2011; 6(11): e27346.

[150] Wang L, Chen S, Xue M, Zhong J, Wang X, Gan L, et al. Homeobox D10 gene, a candidate tumor suppressor, is downregulated through promoter hypermethylation and associated with gastric carcinogenesis. Molecular Medicine. 2012; 18(1): 389.

[151] Chang X, Li Z, Ma J, Deng P, Zhang S, Zhi Y, et al. DNA methylation of NDRG2 in gastric cancer and its clinical significance. Digestive Diseases and Sciences. 2013; 58(3): 715-23.

[152] Wang Y, Li J, Cui Y, Li T, Ng KM, Geng H, et al. CMTM3, located at the critical tumor suppressor locus 16q22. 1, is silenced by CpG methylation in carcinomas and inhibits tumor cell growth through inducing apoptosis. Cancer Research. 2009; 69(12): 5194-201.

[153] Yu J, Cheng YY, Tao Q, Cheung KF, Lam CN, Geng H, et al. Methylation of protocadherin 10, a novel tumor suppressor, is associated with poor prognosis in patients with gastric cancer. Gastroenterology. 2009; 136(2): 640-51.

[154] Cheng Y, Yu J, Wong Y, Man E, To K, Jin V, et al. Frequent epigenetic inactivation of secreted frizzled-related protein 2 (SFRP2) by promoter methylation in human gastric cancer. British Journal of Cancer. 2007; 97(7): 895.

[155] Do Jee C, Kim MA, Jung EJ, Kim J, Kim WH. Identification of genes epigenetically silenced by CpG methylation in human gastric carcinoma. European Journal of Cancer. 2009; 45(7): 1282-93.

[156] Peng D-F, Hu T-L, Schneider BG, Chen Z, Xu Z-K, El-Rifai W. Silencing of glutathione peroxidase 3 through DNA hypermethylation is associated with lymph node metastasis in gastric carcinomas. PLoS One. 2012; 7(10): e46214.

[157] Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, et al. E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. JNCI: Journal of the National Cancer Institute. 2000; 92(7): 569-73.

[158] Guo W, Dong Z, Guo Y, Chen Z, Kuang G, Yang Z. Aberrant methylation of the CpG island of HLTF gene in gastric cardia adenocarcinoma and dysplasia. Clinical Biochemistry. 2011; 44(10): 784-8.

[159] Wani M, Afroze D, Makhdoomi M, Hamid I, Wani B, Bhat G, et al. Promoter methylation status of DNA repair gene (hMLH1) in gastric carcinoma patients of the Kashmir valley. Asian Pacific Journal of Cancer Prevention. 2012; 13(8): 4177-81.

[160] Ling Z-Q, Tanaka A, Li P, Nakayama T, Fujiyama Y, Hattori T, et al. Microsatellite instability with promoter methylation and silencing of hMLH1 can regionally occur during progression of gastric carcinoma. Cancer Letters. 2010; 297(2): 244-51.

[161] Hibi K, Sakata M, Yokomizo K, Kitamura Y-H, Sakuraba K, Shirahata A, et al. Methylation of the MGMT gene is frequently detected in advanced gastric carcinoma. Anticancer Research. 2009; 29(12): 5053-5.

[162] Schneider BG, Peng DF, Camargo MC, Piazuelo MB, Sicinschi LA, Mera R, et al. Promoter DNA hypermethylation in gastric biopsies from subjects at high and low risk for gastric cancer. International Journal of Cancer. 2010; 127(11): 2588-97.

[163] Wu CS, Lu YJ, Li HP, Hsueh C, Lu CY, Leu YW, et al. Glutamate receptor, ionotropic, kainate 2 silencing by DNA hypermethylation possesses tumor suppressor function in gastric cancer. International Journal of Cancer. 2010; 126(11): 2542-52.

[164] Kaneda A, Wakazono K, Tsukamoto T, Watanabe N, Yagi Y, Tatematsu M, et al. Lysyl oxidase is a tumor suppressor gene inactivated by methylation and loss of heterozygosity in human gastric cancers. Cancer research. 2004; 64(18): 6410-5.

[165] Guo W, Dong Z, He M, Guo Y, Guo J, Chen Z, Yang Z, Kuang G. Aberrant methylation of thrombospondin-1 and its association with reduced expression in gastric cardia adenocarcinoma. BioMed Research International. 2010 Mar 15.

[166] Xiong H-L, Liu X-Q, Sun A-H, He Y, Li J, Xia Y. Aberrant DNA methylation of P16, MGMT, hMLH1 and hMSH2 genes in combination with the MTHFR C677T genetic

polymorphism in gastric cancer. Asian Pacific Journal of Cancer Prevention. 2013; 14(5): 3139-42.

[167] Kim Y-K, Yu J, Han TS, Park S-Y, Namkoong B, Kim DH, et al. Functional links between clustered microRNAs: suppression of cell-cycle inhibitors by microRNA clusters in gastric cancer. Nucleic Acids Research. 2009; 37(5): 1672-81.

[168] Yuasa Y, Nagasaki H, Akiyama Y, Hashimoto Y, Takizawa T, Kojima K, et al. DNA methylation status is inversely correlated with green tea intake and physical activity in gastric cancer patients. International Journal of Cancer. 2009; 124(11): 2677-82.

[169] Chen Q, Chen X, Zhang M, Fan Q, Luo S, Cao X. miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. Digestive Diseases and Sciences. 2011; 56(7): 2009-16.

[170] Xu Y, Deng Y, Yan X, Zhou T. Targeting miR-375 in gastric cancer. Expert Opinion on Therapeutic Targets. 2011; 15(8): 961-72.

[171] Wei B, Song Y, Zhang Y, Hu M. microRNA-449a functions as a tumor-suppressor in gastric adenocarcinoma by targeting Bcl-2. Oncology Letters. 2013; 6(6): 1713-8.

[172] Deng H, Guo Y, Song H, Xiao B, Sun W, Liu Z, et al. MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer. Gene. 2013; 518(2): 351-9.

[173] Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, et al. MicroRNA–182 targets cAMP–responsive element–binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. The FEBS Journal. 2012; 279(7): 1252-60.

[174] Xu L, Wang F, Xu X-F, Mo W-H, Xia Y-J, Wan R, et al. Down-regulation of miR-212 expression by DNA hypermethylation in human gastric cancer cells. Medical Oncology. 2011;28(1):189-96.

[175] Liang S, He L, Zhao X, Miao Y, Gu Y, Guo C, et al. MicroRNA let-7f inhibits tumor invasion and metastasis by targeting MYH9 in human gastric cancer. PLoS One. 2011; 6(4): e18409.

[176] Li P, Chen X, Su L, Li C, Zhi Q, Yu B, et al. Epigenetic silencing of miR-338-3p contributes to tumorigenicity in gastric cancer by targeting SSX2IP. PLoS One. 2013; 8(6): e66782.

[177] Tsai K-W, Liao Y-L, Wu C-W, Hu L-Y, Li S-C, Chan W-C, et al. Aberrant hypermethylation of miR-9 genes in gastric cancer. Epigenetics. 2011; 6(10): 1189-97.

[178] Wu H, Huang M, Lu M, Zhu W, Shu Y, Cao P, et al. Regulation of microtubule-associated protein tau (MAPT) by miR-34c-5p determines the chemosensitivity of gastric cancer to paclitaxel. Cancer Chemotherapy and Pharmacology. 2013; 71(5): 1159-71.

[179] Zhu W, Xu H, Zhu D, Zhi H, Wang T, Wang J, et al. miR-200bc/429 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP. Cancer Chemotherapy and Pharmacology. 2012; 69(3): 723-31.

[180] Kwon OH, Park JL, Baek SJ, Noh SM, Song KS, Kim SY, et al. Aberrant upregulation of ASCL2 by promoter demethylation promotes the growth and resistance to 5−fluorouracil of gastric cancer cells. Cancer Science. 2013; 104(3): 391-7.

[181] de Souza CRT, Leal MF, Calcagno DQ, Sozinho EKC, do Nascimento Borges B, Montenegro RC, et al. MYC deregulation in gastric cancer and its clinicopathological implications. PloS One. 2013; 8(5): e64420.

[182] Yashiro M, Yasuda K, Nishii T, Kaizaki R, Sawada T, Ohira M, et al. Epigenetic regulation of the embryonic oncogene ERas in gastric cancer cells. International Journal of Oncology. 2009; 35(5): 997-1003.

[183] Dong W, Wang L, Chen X, Sun P, Wu Y. Upregulation and CpG island hypomethylation of the TRF2 gene in human gastric cancer. Digestive Diseases and Sciences. 2010; 55(4): 997-1003.

[184] Balassiano K, Lima S, Jenab M, Overvad K, Tjonneland A, Boutron-Ruault MC, et al. Aberrant DNA methylation of cancer-associated genes in gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC–EURGAST). Cancer Letters. 2011; 311(1): 85-95.

[185] Niinuma T, Suzuki H, Nojima M, Nosho K, Yamamoto H, Takamaru H, Yamamoto E, Maruyama R, Nobuoka T, Miyazaki Y, Nishida T. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. Cancer Research. 2012; 72(5): 1126-36.

[186] He L-J, Cai M-Y, Xu G-L, Li J-J, Weng Z-J, Xu D-Z, et al. Prognostic significance of overexpression of EZH2 and H3k27me3 proteins in gastric cancer. Asian Pacific Journal of Cancer Prevention. 2012; 13(7): 3173-8.

[187] Sakuraba K, Yokomizo K, Shirahata A, Goto T, Saito M, Ishibashi K, et al. TIP60 as a potential marker for the malignancy of gastric cancer. Anticancer Research. 2011;31(1):77-9.

[188] Qinyu L, Long C, Zhen-dong D, Min-min S, Wei-ze W, Wei-ping Y, et al. FOXO6 promotes gastric cancer cell tumorigenicity via upregulation of C−myc. FEBS letters. 2013; 587(14): 2105-11.

[189] Rotkrua P, Akiyama Y, Hashimoto Y, Otsubo T, Yuasa Y. MiR−9 downregulates CDX2 expression in gastric cancer cells. International Journal of Cancer. 2011;129(11):2611-20.

[190] Gigek CO, Lisboa LCF, Leal MF, Silva PNO, Lima EM, Khayat AS, et al. SMARCA5 methylation and expression in gastric cancer. Cancer Investigation. 2011; 29(2): 162-6.

[191] Yang Q ZR, Sui PC, He HT, Ding L. . Dysregulation of non-coding RNAs in gastric cancer. World Journal of Gastroenterology. 2015; 21(39): 10956.

[192] Tang G, M. Tang, and Y. Xie, . J The role of miRNAs in gastric cancer. Gastroint Dig Syst. 2013; 3(129): 2069-161.

[193] Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, Li E, et al. Suppression of intestinal neoplasia by DNA hypomethylation. Cell. 1995; 81(2): 197-205.

[194] Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell. 1980; 20(1): 85-93.

[195] Fandy TE, Herman JG, Kerns P, Jiemjit A, Sugar EA, Choi S-H, et al. Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. Blood. 2009; 114(13): 2764-73.

[196] Rius M, Lyko F. Epigenetic cancer therapy: rationales, targets and drugs. Oncogene. 2012; 31(39): 4257-65.

[197] Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. Journal of Clinical oncology. 2002; 20(10): 2429-40.

[198] Kantarjian H, Issa JPJ, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, et al. Decitabine improves patient outcomes in myelodysplastic syndromes. Cancer. 2006; 106(8): 1794-803.

[199] Issa J-PJ, Kantarjian HM. Targeting DNA methylation. Clinical Cancer Research. 2009; 15(12): 3938-46.