# Epigenetic regulation and cancer (Review)

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**Abstract.** 'Epigenetics' is defined as the inheritable changes in gene expression with no alterations in DNA sequences. Epigenetics is a rapidly expanding field, and the study of epigenetic regulation in cancer is emerging. Disruption of the epigenome is a fundamental mechanism in cancer, and several epigenetic drugs have been proven to prolong survival and to be less toxic than conventional chemotherapy. Promising results from combination clinical trials with DNA methylation inhibitors and histone deacetylase inhibitors have recently been reported, and data are emerging that describe molecular determinants of clinical responses. Despite significant advances, challenges remain, including a lack of predictive markers, unclear mechanisms of response and resistance, and rare responses in solid tumors. Preclinical studies are ongoing with novel classes of agents that target various components of the epigenetic machinery. In the present review, examples of studies that demonstrate the role of epigenetic regulation in human cancers with the focus on histone modifications and DNA methylation, and the recent clinical and translational data in the epigenetics field that have potential in cancer therapy are discussed.

**Contents**



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## 1. Epigenetic mechanisms

In the eukaryotic nucleus, DNA is compacted into a chromatin structure with the nucleosome as the basic unit, in which histone octamer is surrounded by the 147 bases of DNA for 1.7 laps. The histone octamer includes two elements of the core histone (H3, H4, H2A and H2B) (1). The packaging of DNA into chromatin presents a potential barrier to factors that require DNA as their template. There are mainly three modifications regulating chromatin structure and epigenetic mechanisms of gene expression, including DNA methylation, histone covalent modification and microRNAs (miRNAs) (2). These modifications jointly constitute the 'Epigenetic code' to modulate the expression of the mammalian genome in different cell types, through developmental stages and in diverse disease states including cancer (2-4).

## 2. DNA methylation

DNA methylation is a widespread modification in bacteria, plants and mammals, and this covalent molecular transformation is a natural modification of DNA. DNA methylation which is produced during DNA replication is considered as a stable gene-silencing mechanism. In eukaryotic cells, DNA methylation is the covalent modification taking place at the 5' end of the CpG dinucleotide of the cytosine ring and with S-adenosyl-methionine as its methyl donor. This reaction is catalyzed by the DNMT family, including DNMT1, DNMT3A and DNMT3B. During the process of embryo formation, DNMT3A and DNMT3B are required for DNA methylation from scratch, while DNMT1 is considered to be the methyltransferase maintaining the methylation status (5).

This covalent modification can inhibit the activity of gene transcription; either by blocking the combination of a transcription factor and its binding sites (6), or through recruitment of methylated binding domain proteins that mediate inhibition of gene expression (7). In mammalian cells, DNA methylation occurs mainly in CpG dinucleotides (8). However, CpG sites are not randomly distributed in the genome, but are concentrated in short CpG-rich DNA fragments or DNA fragments in the long repeat so-called 'CpG islands' (8,9). Although for normal cells, the majority of CpG sites of the genome are methylated, usually the cytosine in CpG islands is not methylated in the development and differentiation of tissues. However, in normal cells, certain subsets of CpG islands at the promoter can be

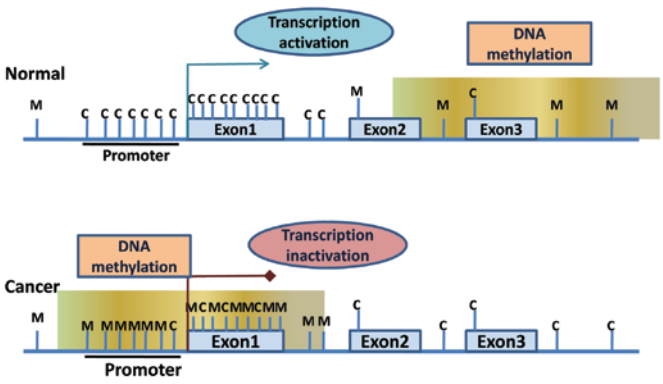


Figure 1. Global changes in DNA methylation in both normal and cancer cells. In normal cells, CpG islands in active promoters are not methylated, thus allowing transcriptional activation. CpG islands within coding regions are often methylated. Reverse patterns are observed in cancer cells.

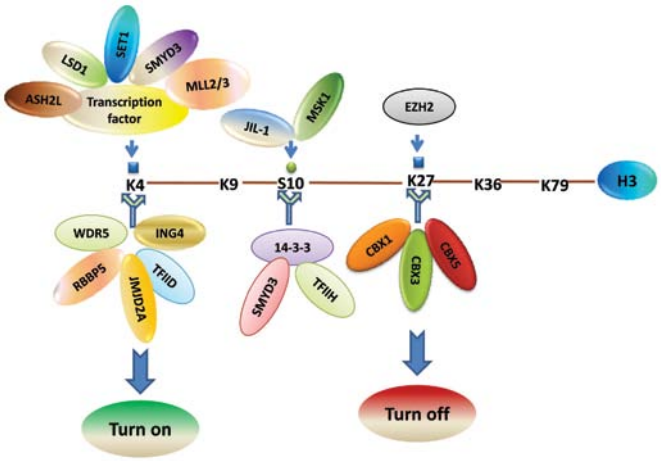


Figure 2. After transcription factor binding to the gene promoter, a series of histone modification enzymes responsible for H3K4me3 are recruited to these nucleosomes, thus catalyzing trimethylation of histone H3 lysine 4. This modification provides binding sites for effector proteins, such as WDR5 and ING4. Subsequently, gene transcription is turned on. However, H3K27me3 mediated by EZH2 leads to transcription inactivation through interaction with CBX1, 3 and 5.

methylated leading to long-term silencing of transcription. The DNA methylation pattern is formed during cell differentiation, but it also causes cells to partially or completely lose the ability to divide. DNA methylation profiles are tissue-specific, and the functions of methylation profiles in different cells are not the same. CpG island-containing gene promoters are usually unmethylated in normal cells to maintain euchromatic structure, which is the transcriptional active conformation allowing gene expression. However, during cancer development, many of these genes are hypermethylated at their CpG island-containing promoters to inactivate their expression by changing open euchromatic structure to compact heterochromatic structure (Fig. 1).

### 3. Histone modification

Histones including H2A, H2B, H3 and H4, together form the histone octamer that is the basic structure of nucleosome components (1). N-terminals of histones protrude out of the nucleosome core, and amino acids of N-terminals easily undergo a series of covalent modifications, such as methylation, acetylation, phosphorylation, ubiquitination and sumolation (10,11). Acting individually or in combination, these modifications are believed to encipher inheritable epigenetic programs that encode distinct nucleosome functions such as gene transcription, X-chromosome inactivation, heterochromatin formation, mitosis, and DNA repair and replication (2-4,10). For example, a previous study showed that direct interaction between the chromodomain of Tip60 and histone H3 trimethylated on lysine 9 (H3K9me3) at DSBs activates the acetyltransferase activity of Tip60. Depletion of intracellular H3K9me3 blocks activation of the acetyltransferase activity of Tip60, resulting in defective ATM activation and widespread defects in DSB repair (12). Mechanistically, these functions are mediated either directly by altering nucleosome interactions with chromatin or indirectly by recruiting effector proteins that possess characteristic modules that recognize specific histone modifications in a sequence-dependent manner. The underlying basis of these epigenetic codes resides in the substrate specificity of the enzymes that catalyze the numerous covalent modifications as well as the enzymes that remove these marks to alter the modifications.

Given that chromatin is the physiological template for all DNA-mediated processes, it is not surprising that histone modifications represent an essential component in controlling the structure and/or function of the chromatin, with different modifications yielding distinct functional consequences. Indeed, previous research has shown that site-specific histone modifications correlate well with particular biological functions such as gene transcription (13). For instance, histone H3 lysine 9 acetylation (H3K9ac), H3 serine 10 phosphorylation (H3S10ph), and H3 lysine 4 trimethylation (H3K4me3) are reported to be associated with transcriptional activation (14). Conversely, H3K27me3 and hypoacetylation of H3 and H4 have been shown to be correlated with transcriptional repression. As stated above, ultimately, the functions of histone modifications are uncovered by the recognition of histone code or histone language by particular cellular machinery such as the transcription apparatus (15). Our previous study found that the histone H3S10 phosphorylation mark is catalyzed by mitogen and stress-activated protein kinase 1 (MSK1) and is recognized by a 14-3-3ε/14-3-3γ heterodimer through its interaction with H3K4 trimethyltransferase SMYD3 and the p52 subunit of TFIIH (14) (Fig. 2).

### 4. microRNAs

microRNAs (miRNAs) are endogenous, short, 19-25 nucleotide long, evolutionarily conserved non-coding RNAs, which partially or perfectly match the 3' untranslated regions (3'UTR) of target mRNAs to regulate gene expression by post-transcriptional silencing and/or by the degradation of target mRNAs (16). Bioinformatics and experimental studies have shown that more than 30% of human genes are direct miRNA targets, which implies that miRNAs function in almost all biological processes including cell cycle regulation, cell growth, apoptosis, cell differentiation and stress reactions. In various species, including the human, a growing number of miRNAs have been determined in the past few years. Genome-

Table I. Global histone lysine methylation patterns in cancer.

| Histone modification | Alteration in cancer (expression compared to normal tissues) | Associated cancer | Refs. |
|---|---|---|---|
| H3K4me1 | Decreased<br>Increased upon progression | Prostate, bladder cancer | (58,104) |
| H3K4me2 | Decreased | Lung, kidney, prostate, non-small cell lung carcinoma, hepatocellular carcinoma, breast, pancreatic, adenocarcinoma, renal cancer | (105,106) |
| | Increased upon progression | Prostate | |
| H3K4me3 | Increased | Prostate, renal cancer | (58,104,107,108) |
| | Decreased | Bladder cancer | |
| H3K9me2 | Decreased | Pancreatic adenocarcinoma, prostate, kidney | (58) |
| H3K9me3 | Increased | Gastric adenocarcinomas | (58,109) |
| | Decreased | Prostate | |
| H3K27me3 | Decreased | Breast, ovarian, pancreatic, colon cancer | (105,108,110,111) |
| | Increased | Paragangliomas | |
| H4K20me1 | Decreased | Bladder cancer, | (104) |
| H4K20me3 | Decreased | Lymphomas, colorectal adenocarcinomas, breast carcinomas, bladder cancer, liver cancer, non-small cell lung cancer | (104-106,112) |

wide studies estimate that miRNA genes represent ~1% of the entire genome in different species; this percentage is similar to other large gene families with regulatory functions such as the home-domain transcription factor family (17,18). The number of genes demonstrated to be the targets of miRNAs is growing rapidly. The latest release of the Sanger miRNA Registry currently annotates more than 800 human miRNAs (http://microrna.sanger.ac.uk; release 13.0), yet many more miRNAs are expected to be identified in the future (19). It is not surprising that miRNAs, just like protein-coding genes, have to be tightly regulated in order to contribute to a distinct transcriptome of a normal cell. In cancer, however, miRNAs have been found to be massively deregulated.

The direct interaction between miRNAs and epigenetic mechanisms is believed to be a quite complicated regulatory network (20). On the one hand, expression of miRNAs is tissue-specific, and is subject to fine and strict regulation by epigenetic mechanisms such as DNA methylation and histone modifications (21); on the other hand, in turn, miRNAs can also affect epigenetic mechanisms and regulate gene transcription; the ability to target post-transcriptional gene-silencing (22).

## 5. Epigenetic abnormalities in tumorigenesis and development

Epigenetic mechanisms are required to maintain normal growth and development and gene expression in different organs (23). Abnormal epigenetic regulation may alter gene expression and function which may lead to diseases such as cancer. Human tumors, in essence, are a genetic disease, since during cancer formation, a large number of genes are mutated or abnormally activated (24,25). However, recent

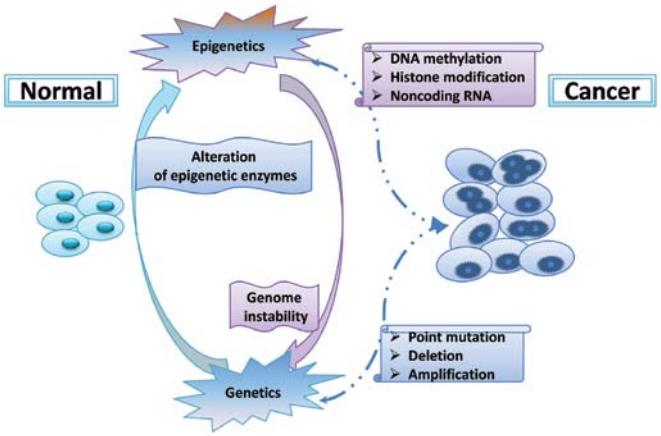


Figure 3. During cancer formation, a large number of epigenetic modifiers are mutated or abnormally activated. At the same time, epigenetic changes such as DNA methylation, histone modifications and microRNAs lead to abnormal gene expression which evoke genome instability.

studies indicate that carcinogenesis cannot be accounted for by genetic alterations alone, but also involve epigenetic changes such as DNA methylation, histone modifications and microRNAs (Fig. 3). Global levels of lysine methylations are quite different between cell types and these molecular changes have been considered to be correlated with various types of cancers (Table I). In addition, the lysine methyltransferases and demethylases are reported to be de-regulated in a variety of cancers (Tables II and III). These molecular alterations lead to permanent changes in the patterns of gene expression that regulate the neoplastic phenotype, such as cellular growth and invasiveness. In this part of the present

Table II. Histone lysine methyltransferases implicated in cancer.

| Writer | New name | Alteration in cancer | Associated cancer | Refs. |
|---|---|---|---|---|
| MLL1 | KMT2A | Translocation, amplification, tandem duplication | Human lymphoid and myeloid leukemias, myelodysplastic syndrome | (113,114) |
| Menin | --- | Mutated<br>Reduced expression<br>Increased expression | Multiple endocrine neoplasia type 1 (MEN1)<br>Lung adenocarcinoma<br>Prostate | (115-117) |
| Ash2L | --- | Increased expression<br><br><br><br>Low levels | Squamous cell carcinomas of cervix and larynx, melanoma, rhabdomyosarcoma, breast and colon carcinomas, neuroendocrine carcinomas, pancreatic ductal adenocarcinomas and gastric carcinomas<br>Hepatocellular carcinoma | (118, 119) |
| Ezh2 | KMT6 | Overexpression<br>Mutation | Prostate, breast, follicular and germinal center B cell lymphoma, gallbladder adenocarcinoma | (120-122) |
| Suv39H1 | KMT1A | Overexpression | Colon | (123) |
| SMYD3 | --- | Overexpression | Colon, breast, hepatocellular carcinoma | (124,125) |
| RIZ1 | KMT8 | Mutation/downregulation | Liver, breast and gastric cancer | (126,127) |
| NSD1 | KMT3B | Translocation<br>Mutations<br>Silencing by promoter hypermethylation | Acute myeloid leukemia<br>Sotos syndrome<br>Neuroblastoma and gliomas | (128,129)<br>(130) |
| NSD2 | --- | Translocation<br>Overexpression | Multiple myeloma<br>Multiple tumors | (131,132) |
| NSD3 | --- | Translocation<br>Amplification | Leukemia<br>Breast cancer | (132,133) |
| G9a | KMT1C | Overexpression<br>Hypoxia-mediated upregulation | Hepatocellular carcinomas<br>Gastric cancer, lung cancer | (81,134) |

Table III. Histone lysine demethylases implicated in cancer.

| Eraser | New name | Alteration in cancer | Associated cancer | Refs. |
|---|---|---|---|---|
| LSD1 | KDM1 | Overexpression<br>Low levels | Prostate, neuroblastoma, breast cancer<br>Hepatocellular carcinoma | (119,135) |
| FBXL10 | KDM2B | Mutation<br>Decreased | Lymphoma<br>Brain, glioblastoma multiforme | (136) |
| JMJD2C | KDM4C | Overexpression | Prostate, esophageal squamous cell carcinoma, desmoplastic medulloblastoma, MALT lymphoma | (135,137,138) |
| RBP2 | KDM5A | Overexpression | Gastric cancer | (139) |
| PLU-1 | KDM5B | Overexpression | Breast, prostate, testis, ovary, lung, bladder cancer | (140,141) |
| UTX | KDM6A | Mutations | Multiple myeloma, several cancers, renal cell carcinoma | (142) |
| JMJD3 | KDM6B | Overexpression | Prostate, pancreatic cancer, lymphoma | (143) |

review, we focus on recent discoveries of epigenetic alterations in several types of tumors including breast, prostate, lung and colon cancer.

*Breast cancer.* Global DNA hypomethylation is frequently reported in breast tumors, but the number of hypomethylated genes is relatively small. DNA hypomethylation of *FEN1*,

*BCSG1*, *PLAU*, *IGF2* and *CDH3* has been detected in breast cancer cells (26,27). However, more than 100 genes have been considered to be hypermethyated in breast cancer, and these aberrantly methylated genes play critical roles in all types of cell processes including cell-cycle regulation, apoptosis, tissue invasion and metastasis, angiogenesis and hormone signaling (28). For instance, *CCND2* and *p16ink4A*/*CDKN2A* which function as crucial regulators of the cell cycle are commonly found to be methylated in breast cancer (29); *APC*, *TWIST* and *HOXA5* which play key roles in apoptosis are silenced due to DNA hypermethylation (30,31); ERα and PR which are critical in hormone regulation are also frequently methylated (32). In addition to protein-coding genes, recent research shows that microRNAs with tumor-suppressor function could be silenced in breast cancer cells through DNA methylation (33). These findings strongly indicate that DNA hypermethylation plays a crucial role in breast carcinogenesis, which cooperatively and synergistically interact with other genetic alterations to promote the development of breast cancer.

A growing number of histone modifications and histone modification enzymes have been found to be deregulated in breast cancer. H4K16ac and its responsible enzyme hMOF were found to be markedly reduced in primary breast carcinomas and medulloblastomas (34). EZH2, which is a subunit of the polycomb-repressive complex 2 (PRC2) and catalyzes the trimethylation of histone H3 on Lys 27 (H3K27), is amplified and overexpressed in breast cancer (35). Furthermore, histone demethylases are shown to function during breast tumorigenesis. Pygo2 associates with histone-modifying enzymatic complexes, specifically the MLL2 histone methyltransferase (HMT) and STAGA histone acetyltransferase (HAT) complexes, to facilitate their interaction with β-catenin and to augment Wnt1-induced, TCF/LEF-dependent transcriptional activation in breast cancer cells (36). Depletion of H3K9 trimethyl demethylase JMJD2B, which is shown to be an integral component of the H3K4-specific methyltransferase, the mixed-lineage leukemia (MLL) 2 complex, impairs the estrogen-induced G (1)/S transition of the cell cycle *in vitro* and inhibits breast tumorigenesis *in vivo* (37). Previous results demonstrate that LSD1 is downregulated in breast carcinomas and that its expression level is negatively correlated with that of TGFβ1 which inhibits the invasion of breast cancer cells *in vitro* and suppresses breast cancer metastatic potential *in vivo* (38).

Recent genome-wide approaches have revealed that miRNAs are globally downregulated in breast cancer. They identified 29 differentially expressed candidates, of which 15 predictive miRNAs were able to distinguish between breast cancer and normal breast tissue (39). Depletion of the let-7 family (containing at least 11 homologous miRNAs) in breast cancer causes enhanced tumorigenicity and is associated with clinical features, such as PgR status (let-7c), a positive lymph node status (let-7f-1, let-7a-3 and let-7a-2), or a high proliferation index (let-7c and let-7d) (40,41). In addition, miR-15/16 is shown to be downregulated in breast cancer which leads to aberrant expression of BCL2 (42). AIB1 which plays an important role in the ERα signaling pathway is overexpressed in breast cancer due to downregulation of miR-17-5p (43). However, certain miRNAs are found to be frequently amplified in breast cancer; for example, miR-21, whose overexpression in breast cancer confers increased invasive capacities and promotes tumor metastasis to the lung (44). Decreased Dicer expression was recently observed in breast cancer, where loss of expression represented an independent prognostic factor for metastatic disease, and reduced expression of Dicer was associated with the highly aggressive mesenchymal phenotype.

*Prostate cancer.* Prostate cancer is the most common cancer in men in Western countries and its incidence is increasing steadily worldwide. Genome-wide DNA hypomethylation has been observed in prostate cancer cells, which may lead to structural and functional changes of the genome. It has been reported that global hypomethylation is considerately lower in patients with metastatic prostate cancer in contrast to non-metastatic prostate cancer (45,46). Gene-specific hypomethylation has also been found in prostate cancers and functions during a variety of cellular processes, such as tumor invasion and metastasis (urokinase plasminogen activator, cellular proliferation gene heparanase) (47), cell cycle control (cancer/testis antigen)(48), hydroxylation of estrogens and activation of carcinogens (cytochrome P450 1B1) (49), X-chromosome inactivation [X (inactivate)-specific transcript] (50). DNA hypermethylation has been the most common and best-characterized epigenetic event in cancer, including prostate cancer. In prostate cancer, a large number of genes have been found to be hypermethylated. These genes are involved in a variety of biological processes including DNA damage repair (Glutathione S transferase P1) (51), signal transduction (RASSF1A) (52), adhesion (E-cadherin, CD44 and galectins) (53), hormonal responses (retinoic acid receptor, androgen receptor and estrogen receptor), apoptosis (death-associated protein kinase) (54), invasion and metastasis (tissue inhibitors of metalloproteinases and galectins) (55) and cell cycle control (cyclins, cyclin-dependent kinases) (56).

Research indicates that alterations of histone modifications play crucial roles during prostate tumorigenesis (57). The increased active histone modifications in prostate cancer facilitate activation of proto-oncogenes and other genes involved in cell growth and survival, while increased repression of histone modifications leads to tumor-suppressor gene silencing. For instance, H3K4me1 and H3K4me2 are found to be increased at the AR enhancers of cell cycle genes (e.g. CDK1), which facilitates upregulation of these cell cycle genes to promote cellular growth (58). H3K4me3 is shown to be enriched in prostate cancer cells, and is correlated with activation of genes involved in cell growth and survival (e.g. BCL2) (59). H3K9me1, H3K9me2 and H3K9me3 have been involved in repression of AR target genes in LNCaP cells (58). In addition, H3K27me3 enrichment at the promoters of genes (e.g., tumor-suppressor genes GAS2, PIK3CG and ADRB2) in metastatic prostate cancer represses the expression of these genes, leading to prostate cancer cell growth, survival and invasion (60).

More than 50 miRNAs have been found to be aberrantly regulated in prostate cancer, including upregulation of several oncogenic miRNAs (miR-488, miR-15a/16, miR-221/-222, miR-21, miR-125b, miR-32, miR-26a, miR-196a, miR-181a, miR-25, miR-93, miR-92 and let-7i) (61) and downregulation of various tumor-suppressor miRNAs (miR-101, miR-126,

miR-205, miR-31, miR-146a, miR-330, miR-34 cluster, miR-218, miR-128, miR-203 and miR-200 family) (62). In prostate cancer cells and primary tumor cells, the cell cycle inhibitor p27Kip1 was found to be extensively downregulated by extra introduction of miR-221/miR-222, which strongly increased cell growth potential by inducing a G1-S shift in the cell cycle subsequently enhancing tumorigenicity in SCID mice (63). In prostate cancer, miR-21 was found to be elevated in PC3 and DU145 cells. Blocking miR-21 by antisense oligonucleotides did not affect proliferation, but it sensitized cells to staurosporine-induced apoptosis and impaired cell motility and invasion (64). Both miR-143 and -145 have been reported to be associated with bone metastasis of prostate cancer and are involved in the regulation of EMT (65). H3K27me3 methyltransferase EZH2 is shown to be enriched due to miR-101 decrease during prostate cancer progression, thus, leading to widespread gene silencing. miR-34 activation can recapitulate the elements of p53 activity, inducing cell cycle arrest and apoptosis by the down-modulation of proteins such as CDK4, CDK6, cyclin D1, cyclin E2, E2F3 and BCL2 (66,67). Notably, miR-34 also inhibits SIRT1, a gene that hinders p53-dependent apoptosis, promoting survival under genotoxic and oxidative stress. Likewise, by targeting glutaminase, miR-23 has been found to participate in the pro-tumorigenic network resulting from MYC overexpression, which is thought to be the most common alteration in prostate cancer. In addition to belonging to the group of reduced miRs, the contribution of miR-146 to prostate cancer progression has been identified in its capacity to repress ROCK1 expression, a downstream effector of hyaluronan-mediated signaling on the CD168 receptor (68).

*Lung cancer.* Lung cancer is a major worldwide health threat and is the leading cause of cancer-related mortality. Global hypomethylation and regional hypermethylation in normally unmethylated CpG islands have all been implicated in lung cancer (69). Loss of imprinting of the H19, IGF2 and MEST genes has been found in lung cancer cells due to genome-wide DNA hypomethylation, which may result in deregulated cell growth. In addition, upregulation of cancer testis antigens (CTAs) including the melanoma-associated antigen family as a result of global hypomethylation has also been observed (70). However, a number of tumor-suppressor genes has been shown to be aberrantly methylated and associated with different cellular processes, such as cell cycle regulation (p16) (71), DNA repair (MGMT) (72), apoptosis (DAPK, caspase 8, ARF, FAS and TRAILR1) (72,73), RAS signaling (RASSF1A, NORE1A and G0S2) and invasion (cadherins, TIMP3 and laminin family) (74,75).

Different histone modifications may play crucial roles in the epigenetic alterations in lung cancer. Gain of H4K5ac and H4K8ac and loss of H4K12ac, H4K16ac and H4K20me3 have been found in lung cancer cells (76). In addition, low cellular levels of both H3K4me2 and H3K18ac predict poor clinical outcome in lung cancer patients (77). HDACs have been reported to repress critical gene pathways involved in protection against lung cancer and, therefore, reduction in lung HDACs may promote tumorigenesis (78). Previous studies have demonstrated that expression of HDACs is significantly increased in various lung cancer cells and is associated with poor prognosis after surgery. The abnormal overexpression of HDACs may result in the downregulation of critical tumor-suppressor genes which promotes tumorigenesis. For example, transcription factor ZBP-89, which has been implicated in the induction of growth arrest and apoptosis, can recruit HDAC3 to the promoter of p16, and thus downregulates p16 expression by altering the histone modification status. In addition, FEZ1 and MYO18B have been suggested to be related to tumorigenesis of lung cancer through repression as the result of histone deacetylation (79,80). In addition to DNA methylation, alteration of histone modification is another crucial mechanism leading to the silencing of TGF β RII, MAGE-3, Ep-CAM and MYO18B (81). These results suggest that histone deacetylation contributes to gene silencing in lung cancer cells and is involved in lung carcinogenesis.

Previous studies have demonstrated that miRNA alterations occur as an early event in response to environmental carcinogens ahead of the onset of cancer. The expression of let-7 miRNA, which correlates with shorter survival and is an independent prognostic factor, is observed to be reduced in primary lung tumors. It has also been observed that overexpression of miRNA let-7 in A549 lung adenocarcinoma cell lines inhibited cancer cell growth. Further studies have shown that let-7 negatively regulates the expression of RAS and MYC by targeting their mRNAs for translational repression (82,83). Downregulation of miR-128b, which is a direct negative regulator of the EGFR oncogene, is found in lung tumors. In addition, expression of miR-124a is epigenetically silenced by DNA hypermethylation in lung cancer (84). In contrast to the above miRNAs, the expression of miRNA cluster miR-17 is markedly amplified in lung cancer, and stimulates cell proliferation. The predicted targets of the miR-17 cluster include PTEN, E2F1 and RB2 that are known to play important roles in lung cancer (85). In addition, abnormal amplifications of miR-155 and miR-21 have been correlated with poor prognosis and reduced survival of patients diagnosed with lung cancer (86).

*Colon cancer.* Colon cancer is one of the most common types of cancer and is a leading cause of cancer-related mortality worldwide. It has been more than 25 years since an extensive loss of DNA hypomethylation was reported in colon cancer cells. Various studies have confirmed this initial finding not only in colon cancer but also in a number of other cancer types. This widespread hypomethylation may include different epithelial cells, increasing genome instability, overexpression of a number of genes and loss of imprint of specific genes. Hypermethylation targeting promoters of specific genes has also frequently been detected in colon cancer. Numerous genes influenced by DNA hypermethylation are correlated with diverse biological functions including cell cycle control (p16, p15, MINT1, MINT2 and MINT31), DNA damage repair (MLH1, MSH2 and MGMT), apoptosis (DAPK), tumor cell invasion (APC and LKB1), cell proliferation (IGF2) and tumor angiogenesis and metastasis (COX-2) (87-89).

Histone modifications are necessary for the regulation of gene expression, but levels of these covalent changes and modification enzymes are usually altered in colon cancer. Colon cancer cells exhibit increased HDAC activity compared with non-malignant cells. HDACs are upregulated in colon cancer cells and in primary colon cancer. For example, over-

expression of HDAC1 and HDAC3 may silence SLC5A8, the gene coding for the Na(+)-coupled pyruvate transporter (90); upregulation of HDAC1 may repress P21, the gene involved in cell cycle regulation (91); and amplification of HDAC3 which is determined in approximately half of all colon adenocarcinomas alters the epigenetic programming of colon cancer cells to impact intracellular wnt signaling and their sensitivity to external growth regulation by vitamin D (92). In addition, YPEL3 and NDRG1, members of the secreted frizzle-related proteins (SFRPs) and the GATA family of transcription factors which are demonstrated to be silenced in specific colon cancer cell lines are occupied by inactive histone modifications (93,94). Enrichment of H3K27me3, HDAC1 and EZH2 are found at the promoters of RUNX3 and PTPRR-1 in cancer cells, which may downregulate these genes and are associated with tumor progression (95). It has also been reported that overexpression of hSET1 in colon cancer promotes cell proliferation and cancer cell survival (96). Furthermore, HIF recruits JMJD1A to regulate the expression of adrenomedullin (ADM) and growth and differentiation factor 15 (GDF15), ultimately enhancing tumor growth (97). In addition, RGC-32 may contribute to the development of colon cancer by regulating chromatin assembly (98).

miRNAs are negative regulators of target genes through post-transcriptional inhibition of specific mRNAs. Both overexpression and suppression of miRNAs have been found to be involved in the tumorigenesis of colon cancer. Overexpressed miRNAs such as miR-20, miR-21, miR-17-5p, miR-15b, miR-181b, miR-191 and miR-200c have been found in colon cancer cells. miRNAs function by targeting and inhibiting different tumor-suppressor genes such as E2F1, tropomyosin 126, PTEN and Pdcd4 (99). Lower levels of mature miRNAs such as let7, miR-22, miR-34a, miR-126, miR-143, miR-145, miR-342 and miR-345 are also found in colon cancers, suggesting that they act as tumor-suppressor miRNAs (100). The loss of such miRNAs may lead to overactivity of oncogenes and deregulation of signaling pathways finally promoting cell growth and invasion in colon cancer. For example, the putative identified targets of miR-145 are transforming growth factor receptor II and insulin receptor substrate 1 (IRS-1), which promote tumor-suppressor activity (101). Repression of miR-22 upregulates HIF-1α expression, promoting VEGF production during hypoxia (102). miR-345 may play an important antineoplastic role; a growth inhibitor in the development of colon cancer through downregulation of BCL2-associated athanogene 3 (BAG3) (103).

## 6. Epigenetic therapy and future challenges

Human tumors are a group of diseases triggered by various causes, including progressive genetics and abnormal epigenetics. More and more studies have demonstrated that epigenetic changes are main factors in tumorigenesis and cancer development. Epigenetic abnormalities occurring in tumors have led to the development of epigenetic treatment in cancer. Epigenetic therapy aims to reverse the epigenetic alterations occurring in tumors, thus, restoring the normal epigenome.

Remarkable progress has been made during the past few decades on DNA methylation and histone modifications in gene transcription, yet the role of epigenetic events in cancer has not been fully explained. However, great progress has been accomplished in regards to epigenetic drugs targeting chromatin and histone-modifying enzymes. Many epigenetic drugs, including two DNA methyltransferase enzyme (DNMT) inhibitors and a deacetylase (HDACs) inhibitor have been approved by the FDA as effective drugs for cancer treatment. Meanwhile, various inhibitor drugs, such as FK228, SAHA and MS-275, have already been the focus of phase III clinical experiments. Nevertheless, there is still a long way to go until the succesful epigenetic treatment of cancer. The main strategy of recent epigenetic treatment is to inhibit abnormal DNMTs and HDACs using specific inhibitors. More specific and effective inhibitors should be developed to reduce unwanted side-effects as much as possible since epigenetic modifying enzymes function in a wide range of organs in the body; in addition, epigenetic changes occurring in tumors have not been completely studied. Research on detailed epigenetic changes in cancer, and the in-depth study of tumor pathology are expected to enhance the ability to diagnose and treat cancer.

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