EPIGENETIC EVENTS IN TUMORIGENESIS:
PUTTING THE PIECES TOGETHER

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The development of cancer is a complex multifactorial process traditionally viewed as the stepwise accumulation of genetic alterations. However, recent advances in field of cancer research have established that all major human cancers, in addition to a large number of genetic alterations, exhibit prominent epigenetic abnormalities. This review presents current evidence that epigenetic alterations are not only key features of cancer cells, but they also may be key events in the initiation of carcinogenesis. The early appearance of cancer-linked epigenetic changes that are similar to those found in malignant cells provides a unique opportunity to use them as biomarkers in early cancer detection, indicators of carcinogenic exposure, and in the assessment of the carcinogenic potential of environmental chemical and physical agents.

Key Words: epigenetics, DNA methylation, histone methylation, carcinogenesis.

Classically, the development of cancer in humans has been viewed as a progressive, multistep process of transformation of normal cells into malignant cells driven by genetic alterations [1, 2]. However, a wealth of data in the past decade indicates that human cancer cells, in addition to a large number of genetic alterations, exhibit prominent epigenetic abnormalities, e.g. aberrant DNA methylation and histone modifications. This knowledge has largely changed the view on cancer as being a solely genetic disease [3]. Currently, cancer is recognized as a disease associated with both genetic and epigenetic alterations, and both of these components cooperate and complement each other to promote cancer progression [4]. Recognition of the fundamental role of epigenetic alterations in cancer progression has resulted in the identification of a number of epigenetic abnormalities in cancer that can be used as diagnostic and therapeutic targets for cancer management. While the role of epigenetic changes in cancer has been studied extensively [3, 5, 6], the contribution of epigenetic alterations to the mechanism of neoplastic cell transformation has remained relatively unexplored, despite the fact that the involvement of epigenetic events in cancer initiation has been proposed for forty years [7]. This review summarizes the significance of epigenetic changes in cancer cells and the role of epigenetic dysregulation in carcinogenesis.

OVERVIEW OF EPIGENETICS:
DNA METHYLATION AND HISTONE MODIFICATIONS

«Epigenetics» is defined as heritable changes in gene expression associated with modifications of DNA or chromatin proteins that are not due to any alteration in the primary DNA sequence [4, 6]. Such modifications include the best known and much studied methylation of DNA, modifications of the proteins that bind to DNA, and the nucleosomes positioning along DNA [4].

DNA methylation, a well-known primary epigenetic regulator of gene expression, is the addition of a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the fifth carbon atom in the cytosine pyridine ring, resulting in the formation of 5-methylcytosine (5meC) in DNA. This reaction is catalyzed by DNA methyltransferases (DNMTs) [8, 9]. In eukaryotes, this stable, post-synthetic epigenetic mark is found exclusively at cytosine residues at CpG sequences.

Genomic DNA methylation refers to the overall content of 5meC in the genome. Approximately 70–90% of the CpG dinucleotides in the mammalian genome are methylated; however, the CpG sites are not distributed uniformly across the genome [4, 9, 10]. They are concentrated in short regions (< 4 kb) that contain the high G + C content and the high frequency of CpG dinucleotides called «CpG islands» and long domains that contain predominantly repetitive elements. CpG sites located in CpG islands are undermethylated, while most of the remaining CpG sites are methylated. Promoters and first exons of the majority of genes in the genome are strongly enriched in unmethylated domains and depleted in methylated domains, which are found predominantly in interspersed and tandem repetitive sequences and exons other than first exons [10].

DNA methylation is initiated and established by means of the de novo DNA methyltransferase DNMT3 family (DNMT3A and DNMT3B), whose expression is coordinated by DNMT3L, lymphoid-specific helicase (LSH), microRNAs, and piRNAs. During DNA replication, DNA methylation is maintained by a complex, cooperative interplay of the maintenance methyltransferase DNMT1 along with the UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) protein, de novo DNA methyltransferases DNMT3A and DNMT3B, methyl-CpG-binding protein, and histone-modifying enzymes [8, 9] (Figure).
The unifying feature of neoplastic cells is a profoundly distorted epigenetic landscape that is characterized by global genomic hypomethylation, hypermethylation of critical genes, and altered histone modification patterns [3].

DNA hypomethylation signifies one of the major DNA methylation states, the other being hypermethylation, and in most cases refers to a relative situation in which there is a decrease from the «normal» methylation level. The loss of global DNA methylation, the first epigenetic abnormality identified in cancer cells more than a quarter century ago [15], continues to be a central feature and one of the most common molecular alterations in all major human cancers, including colon, gastric, lung, liver, breast, bladder, and ovarian.

DNA hypermethylation is the opposite state of DNA methylation and refers to a relative increase of methylation at normally undermethylated CpG islands. In the context of epigenetic dysregulation in cancer, hypermethylation of CpG island containing promoters and concomitant inhibition of gene expression is the most frequently and consistently observed epigenetic abnormality in cancer cells and is regarded as a crucial event in cancer progression [3, 5, 16]. For instance, recent findings demonstrate that there are more than one hundred hypermethylated and transcriptionally silenced genes found in breast cancer alone [17], just as in all major human cancers [18].

Silencing of tumor suppressor genes by promoter hypermethylation is not the only mechanism favoring cancer progression. In addition to gene-specific hypermethylation, a number of normally methylated genes undergo progressive hypomethylation accompanied by increased expression in human cancers. Table lists selected hypomethylated and over-expressed genes in various human cancers. This list is a noticeably shorter one when compared to the number of hypermethylated genes in human cancers and even in any specific type of cancer. This is because the number of genes that can potentially be demethylated (normally methylated) is substantially smaller than the number of genes that can potentially be methylated (normally unmethylated), which is directly predetermined by the methylation landscape of the genome. Despite the different number of cancer-linked hypomethylated and hypermethylated genes, the dynamic of gene-specific methylation changes in cancer is identical: the progressive accumulation of hypomethylated and/or hypermethylated alterations during cancer progression.

DNA methylation changes in cancer are not isolated events. They occur in the environment of large-scale disruption of the cellular epigenome and are associated primarily with alteration of histone modifications, especially acetylation, methylation, phosphorylation, biotinylation, and ubiquitylation. Similar to alterations in DNA methylation, changes in histone modifications in cancer cells occur on both the genome-wide and gene-specific scales. With respect to cancer, acetyla-
tion and methylation of histone lysine residues are the best-studied histone modifications to date. Typically, histone acetylation is associated with active transcription, whereas methylation may be associated with either active or repression states depending on the modified site [14, 19]. For example, methylation of histone H3 lysine 4 is associated with increased transcription, while methylation of histone H3 lysine 27 or histone H3 lysine 9 is associated with transcriptional silencing [19]. It has been demonstrated that the hypermethylated promoter CpG islands of many transcriptionally repressed genes are accompanied by either lysine hypoacetylation of histones H3 or H4 and/or by increased H3 lysine 9 and H3 lysine 27 methylation.

Table. Selected list of the hypomethylated genes in human cancers

<table>
<thead>
<tr>
<th>Tumor Types</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>BCSG1, CDH1, CDH3*, NAT1, PEN1, SNCG, PLAU, CAV1, ZEB2, MUC3A, IL-10</td>
</tr>
<tr>
<td>Colon</td>
<td>S100A4, CYP 2W1, CDH3, BAGE, DCN, MAGEA1, MAGEA3, MUC3A</td>
</tr>
<tr>
<td>Uterus</td>
<td>S100A4, PAX2, DNMT3L, CAGE, ESR1</td>
</tr>
<tr>
<td>Stomach</td>
<td>MAGEA1, MAGEA3, XAGE1, CCND2, SERPINB5, MUC2</td>
</tr>
<tr>
<td>Head – Neck</td>
<td>TKT1L</td>
</tr>
<tr>
<td>Leukemia</td>
<td>BCL2, TCL1, PRAME, DDX43</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>TNFRSF8F, LGALS7</td>
</tr>
<tr>
<td>Lung</td>
<td>MUC3A</td>
</tr>
<tr>
<td>Melanoma</td>
<td>TIMP1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>CLDN4, HNF1B, BORIS</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>SERPINB5, TFF3, CLDN4, LCN2, MUC3A</td>
</tr>
<tr>
<td>Prostate</td>
<td>HPSE2, PLAU</td>
</tr>
<tr>
<td>Kidney</td>
<td>C9</td>
</tr>
<tr>
<td>Thyroid</td>
<td>SERPINB5</td>
</tr>
<tr>
<td>Wilms Tumors</td>
<td>GLI1R1/RTVP1</td>
</tr>
</tbody>
</table>

Note: *Genes that were found to be hypomethylated in more than one tumor type

**ROLE OF EPIGENETIC ALTERATIONS IN CARCINOGENESIS**

Recent advances in the field of cancer research suggest that epigenetic alterations, in addition to genetic changes, may be the fundamental events in cancer initiation. The development of cancer is a complex, multifactorial process characterized by many biologically significant and interdependent alterations. One of these changes is epigenetic deregulation. It is clear that cancer, by itself, can trigger epigenetic alterations that reflect the transformed state of neoplastic cells; however, the distortion of the cellular epigenetic status in normal cells can also have an impact on the predisposition to pre-cancer specific pathological states and cancer development. This leads to the suggestion that epigenetic alterations are not only important features of cancer cells, but they also play a major role in the initiation and propagation of cancer. The «epigenetic model of cancer initiation» suggests that epigenetic alterations that occur in stem, progenitor, or differentiated cells are the earliest events in cancer initiation that predispose to mutational events, giving rise to cancer stem cells and contributing to tumor heterogeneity [20, 21]. The results of recent studies demonstrating the early emergence of epigenetically reprogrammed cells, which have epigenetic alterations that are similar to those found in malignant cells, provided strong experimental support to this hypothesis.

DNA hypomethylation is one of the earliest key epigenetic events in the carcinogenic process. However, the functional significance of global DNA hypomethylation in tumorigenesis remains unclear. In some forms of cancer, it has been attributed as a driving force leading to malignant cell transformation. This is supported by strong evidence demonstrating that the decrease of DNA methylation by disruption of DNMT1 [22, 23] and LSH [24] functioning causes tumor induction. In contrast, in other forms of cancer, DNA hypomethylation occurs via a gradual mechanism and is not a requirement for transformation [25, 26] and even more, for some cancers, e.g. gastric and intestinal cancers [27, 28], reduction of DNA methylation suppresses carcinogenic process.

The mechanistic link between the loss of DNA methylation and cancer development is associated with events that destabilize the genome, including induction of mutations [29], chromosomal instability [30], reactivation and transposition of retrotransposable elements [31], loss of imprinting [32], and activation of normally silenced oncogenes [33]. One of the primary functions of DNA methylation is safeguarding the genome by silencing repetitive sequences, such as long interspersed nucleotide elements 1 (LINE-1) and short interspersed nucleotide elements (SINE), retroviral intrachromosomal A particle (IAP), and Alu elements. The methylation landscape of mammalian genomes consist of short unmethylated domains embedded in a matrix of long methylated domains composed of predominantly interspersed and tandem repetitive sequences [10]. Because of this, loss of DNA methylation largely affects only these areas of the genome. This is evidenced by the strong correlation between the loss of global DNA methylation and the demethylation of repetitive sequences during tumorigenesis. There are two well-established consequences associated with the loss of DNA methylation at repetitive sequences that may contribute to tumorigenesis. First, demethylation of repetitive sequences located at centromeric, pericentromeric, and subtelomeric chromosomal regions may cause the induction of chromosomal abnormalities. For example, recent findings have demonstrated that DNA hypomethylation at the centromeric region causes permissive transcriptional activity at the centromere and the subsequent accumulation of small minor satellite transcripts that impairs centromeric architecture and function. Likewise, hypomethylation of the subtelomeric regions is associated with enhanced transcription of the telomeric region. Second, hypomethylation of LINE-1, SINE, Alu, and IAP retroviral elements causes their activation and transposition, which may lead to genomic instability. An integral role of the loss of DNA methylation and the presence of these alterations in the neoplastic process is now commonly accepted.

In addition to the vital role of DNA methylation in the safeguarding of the genome, compelling evidence demonstrates an equally important role of histone modifications for the maintenance of genomic stability. Particularly, loss of histone H3 lysine 9, H3 lysine 27, and H4 lysine 20 trimethylation markedly impairs...
genome stability and has been frequently detected during carcinogenesis [34].

It is well-established that transcriptional silencing of a range of protein-coding genes caused by hypermethylation of promoter CpG islands plays a critical role in carcinogenesis [3, 5, 18]. One of the most compelling examples of the link between epigenetic alterations and genomic instability during carcinogenesis is epigenetic silencing of DNA repair genes [35], resulting in the elevation of mutation rates in critical cancer-related genes. For example, the epigenetic silencing of O^6^-methylguanine-DNA methyltransferase (MGMT) gene leads to a greater mutation rate in K-ras and p53 genes during human colorectal carcinogenesis [36, 37]. Likewise, elevated p53 gene mutation frequency is associated with transcriptional inactivation of the breast cancer susceptibility gene (BRCA1) caused by its promoter hypermethylation in human sporadic breast cancer [38], and microsatellite instability in sporadic colorectal cancer is linked to hypermethylation of the hMLH1 DNA mismatch repair gene [39].

**EPIGENETIC ALTERATIONS, CARCINOGEN EXPOSURE, AND CANCER RISK ASSESSMENT**

Currently, it is accepted that environmental exposure to natural and man-made chemical and physical agents is one of the major causes of human cancer [40]. In a broad sense, carcinogenesis may be induced through either genotoxic or non-genotoxic mechanisms. Environmental agents or chemicals are considered genotoxic if they, or the products of their metabolic activation, interact directly with DNA, causing mutations and leading to tumor formation [41]. Non-genotoxic carcinogens are a diverse group of chemical compounds that are known to cause tumors by mechanisms other than directly damaging DNA [42]. Nonetheless, mounting evidence suggests that despite different mechanisms of action with regards to DNA reactivity both classes of agents were shown to lead to prominent epigenomic alterations in tissues that are targets for carcinogenesis as a result of exposure. Specifically, the results obtained in numerous animal studies have demonstrated that epigenetic alterations are early indicators of genotoxic or non-genotoxic carcinogenic exposure [43–51]. Additionally, considering the stability and inheritance of epigenetic alterations through transmission of carcinogen-induced aberrant epigenetic patterns from one cell generation to another, epigenetic alterations may be better biomarkers of carcinogenic exposure. The results of recent human studies have provided strong support for this suggestion [51–53].

**FUTURE DIRECTIONS AND PERSPECTIVES**

It is becoming increasingly evident that epigenetic alterations are not only key features of cancer cells, but they also may be key events in the initiation of cancer. The early appearance of cancer-linked epigenetic changes that are similar to those found in malignant cells provides a unique opportunity to use them as biomarkers in early cancer detection, indicators of carcinogenic exposure, and in the assessment of the carcinogenic potential of environmental chemical and physical agents. More importantly, the reversibility of epigenetic alterations opens a novel mechanism-based approach not only to cancer treatment but also to prevention of cancer.

**Note.** The views expressed in this paper do not necessarily represent those of the U.S. Food and Drug Administration.

**REFERENCES**
