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Mini-review

DNA methylome alterations in chemical carcinogenesis ☆

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ABSTRACT

Carcinogenesis, a complex multifactorial process of the transformation of normal cells into malignant cells, is characterized by many biologically significant and interdependent alterations triggered by the mutational and/or non-mutational (i.e., epigenetic) events. One of these events, specific to all types of cancer, is alterations in DNA methylation. This review summarizes the current knowledge of the role of DNA methylation changes induced by various genotoxic chemicals (carcinogenic agents that interact with DNA) and non-genotoxic carcinogens (chemicals causing tumor by mechanisms other than directly damaging DNA) in the lung, colorectal, liver, and hematologic carcinogenesis. It also emphasizes the potential role for epigenetic changes to serve as markers for carcinogen exposure and carcinogen risk assessment.

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1. Introduction

It is widely believed that continuous exposure to natural and man-made chemicals and physical agents is a major cause of chronic human diseases, including cancer [1–3]. This suggestion is based on a wealth of data demonstrating that environmental and occupational factors have the ability to affect the proper functioning of key physiological systems in the human body.

In a broad sense, all carcinogenic agents may be divided into genotoxic and non-genotoxic carcinogens based on the mechanism of cancer causation. Genotoxic carcinogens are agents that interact with DNA, either directly or after metabolic activation, leading to a variety of genotoxic alterations, including DNA adduct formation and DNA lesions. However, even within classical genotoxic carcinogenesis models, the formation of DNA adducts and/or damage to DNA is necessary but not sufficient for tumor formation. Non-genotoxic carcinogens are a diverse group of chemicals that are known to cause tumors by mechanisms other than directly damaging DNA.

Traditionally, carcinogenesis research has focused on investigating of various molecular abnormalities, DNA damage, DNA adduct formation, and genetic aberrations in cancer causation and progression, despite the fact that the importance of epigenetic mechanisms in the carcinogenic process was first suggested by James Miller in 1970 [4]. Nonetheless, accumulating evidence suggests that regardless of the different mechanisms of action, both genotoxic and non-genotoxic carcinogens induce prominent epigenetic abnormalities, including global genomic *hypo*methylation, gene-specific DNA *hyper*methylation or *hypo*methylation, abnormal functioning of DNA and histone modifying enzymes, altered histone modification patterns, and aberrant expression of microRNAs, in tissues that are susceptible to carcinogenesis as a result of exposure [5–9].

2. DNA methylation changes in cancer

2.1. Global DNA hypomethylation

DNA methylation, a well-known primary epigenetic regulator of chromatin organization and gene expression, is the addition of a methyl group from the universal methyl donor S-adenosyl-L-methionine (SAM) to carbon five in the cytosine pyridine ring, resulting in the formation of 5-methylcytosine (5mC) in DNA. This reaction is catalyzed by a family of DNA methyltransferases (DNMTs). In mammalian somatic cells, this stable, post-synthetic epigenetic mark is found exclusively at cytosine residues of CpG dinucleotides, while in embryonic stem cells, DNA methylation occurs at both CpG and non-CpG sequences [10]. DNA methylation is initiated and established by means of the *de novo* DNA methyltransferase DNMT3 family (DNMT3A and DNMT3B), the expression of which is coordinated by DNMT3L, lymphoid-specific helicase (Lsh), microRNAs, and piwi-interacting RNAs (piRNAs). During DNA replication, DNA methylation is maintained through a complex, cooperative interaction of the maintenance DNA methyltransferase DNMT1 with the UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1) protein, *de novo*

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

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DNA methyltransferases DNMT3A and DNMT3B, methyl-CpG-binding proteins, and histone-modifying proteins [11–13].

Genomic DNA methylation refers to the overall 5mC content 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 [14]. The highest frequency of CpG dinucleotides and the highest G + C content are found in short unmethylated regions (<4 kb) of DNA called "CpG islands" [15,16]. In normal cells, CpG islands are located in intragenic and intergenic regions, and at the 5′-ends of genes [17]. Of the various types of CpG islands, only those that span the promoter region of genes are predominantly unmethylated [14,17].

DNA hypomethylation signifies one of the two major DNA methylation states and refers to a condition in which there is a decrease in the number of methylated cytosine bases from the "normal" methylation level. The loss of global DNA methylation is one of the most common DNA methylome alterations in human cancers [18]. DNA hypomethylation arises mainly from the loss of methylation at normally heavily methylated areas of genome. The molecular events that lead to hypomethylation of DNA are elusive, and it is likely that multiple pathways are involved. Hypomethylation of DNA can be achieved either passively or actively. Passive loss of methylated cytosines in the genome may be a consequence of (i) a limited availability of SAM, (ii) a compromised integrity of DNA, or (iii) altered expression and/or functioning of the DNA methylation machinery [18]. Recent studies have provided compelling experimental evidence for the existence of another mechanism of DNA demethylation, active loss of DNA methylation, which is associated with the function of DNA repair machinery [19–22].

A mechanistic link between genome demethylation and carcinogenesis is indicated by the several well-established consequences that induce and promote tumor development. First, genomic demethylation causes a significant elevation in mutation rates [23,24] and aberrant activation of "normally" silenced tumor-promoting genes [25]. Second, hypomethylation of DNA results in the loss of genomic imprinting (LOI), which is currently considered as one of the earliest and most frequent alterations in human tumors [26–28]. LOI refers to loss of parent-of-origin-dependent monoallelic gene expression regulation. LOI induced by DNA hypomethylation leads to activation of the normally silent allele of growth-promoting genes, e.g., *IGF2* [29], resulting in a biallelic expression of imprinted genes, or silencing of the normally active allele of growth-inhibitory genes, e.g., $P57^{KIP2}$ [30], resulting in a loss of gene expression. Third, demethylation of repetitive sequences, such as long interspersed nucleotide elements (LINE)-1 and short interspersed nucleotide elements (SINE), retroviral intracisternal A particle (IAP), and Alu elements, located at centromeric, peri-centromeric, and sub-telomeric chromosomal regions, may cause chromosomal abnormalities and genomic instability via the induction of permissive transcriptional activity of repetitive elements. The subsequent accumulation of these transcripts impairs centromeric and/or telomeric architecture and function [31–34].

Several recent reports have established a crucial role of 5-hydroxymethylcytosine (5hmC), an oxidation product of 5mC in cancer [22,35,36]. Specifically, the loss of 5hmC has been found in a broad spectrum of solid tumors, including lung, breast, brain, gastric, and colorectal cancers [37–40]. More importantly, recent reports by Kudo et al. [38] and Yang et al. [39] demonstrated that neoplastic cell transformation and tumor development was accompanied by a decrease of 5hmC levels.

2.2. Cancer-linked gene-specific DNA hypermethylation

DNA hypermethylation is the opposite state of DNA hypomethylation and refers to the relative gain of methylation at normally undermethylated DNA domains. DNA hypermethylation is the most extensively studied epigenetic abnormality in cancer [41], and it has been established unequivocally that hypermethylation of promoter CpG islands causes permanent and stable transcriptional silencing of a wide range of protein-coding genes [42] and non-coding RNA genes [43,44].

One of the most compelling examples of the link between DNA hypermethylation and carcinogenesis is epigenetic silencing of critical tumor-suppressor genes, especially cyclin-dependent kinase inhibitor 2A (*CDKN2A*; $p16^{INK4A}$), secreted frizzled-related protein genes (*SFRPs*), adenomatous polyposis coli (*APC*), Ras association (RalGDS/AF-6) domain family member 1 (*RASSF1A*), and GATA binding protein 4 (*GATA4*). The aberrant silencing of these genes allows abnormal survival and clonal expansion of initiated cells [41]. Additionally, hypermethylation of DNA repair genes, including O^6-methylguanine-DNA methyltransferase (*MGMT*), Xeroderma pigmentosum group C (*XPC*), MutL homolog 1 (*MLH1*), and breast cancer 1 and 2 (*BRCA1* and *BRCA2*) genes, results in insufficient DNA repair and leads to a reduction in genomic stability and various genetic aberrations, and, in particular, the elevation of mutation rates in critical cancer-related genes [45,46]. For example, the epigenetic silencing of *MGMT* leads to greater mutation rates in *KRAS* and *TP53* genes during human colorectal carcinogenesis [47,48]. Likewise, transcriptional inactivation of the *BRCA1* and *MLH1* genes by promoter hypermethylation results in an elevated *TP53* gene mutation frequency in human sporadic breast cancer [49] and microsatellite instability in sporadic colorectal cancer [50].

There is also growing evidence for the importance of non-CpG island-promoter methylation in cancer, including methylation of CpG island shores [51], non-CpG promoters [52,53], and coding regions [54,55], which results in gene silencing.

2.3. Cancer-linked gene-specific DNA hypomethylation

It is well established that in the human genome the majority of gene promoters containing CpG islands are unmethylated; however, the results of several comprehensive studies have indicated that 3–4% of the promoter CpG island-containing genes are methylated and silenced [17,56]. Until recently, an overwhelming number of the studies in the field of cancer research have focused on the role of epigenetically-driven gene silencing as the main mechanism favoring tumor development and progression. This overshadowed the importance of gene-specific hypomethylation in cancer; however, accumulating evidence indicates that the hypomethylation of "normally" methylated CpG island-containing genes also plays a significant role in tumor development. Currently, several hypomethylated tumor-promoting genes, including S100 calcium binding protein A4 (*S100A4*), plasminogen activator, urokinase (*UPA*), heparanase (*HPA*), synuclein, gamma (*SNCG*), trefoil factor 3 (*TFF3*), and flap structure-specific endonuclease 1 (*FEN1*), have been identified in major human cancers [18]. Additionally, a recent report by Fernandez et al. [57] demonstrated that cancer development is accompanied by a progressive loss of CpG methylation in non-CpG-island promoters.

Importantly, the existence of both hypermethylation and hypomethylation cancer-linked gene-specific DNA methylation events that are associated with the well-established hallmarks of cancer, including the acquisition of persistent proliferative signaling, resistance to cell death, evasion of growth suppression, replicative immortality, inflammation, deregulation of energy metabolism, induction of angiogenesis, and activation of invasion [58], complement and enhance each other in the disruption of cellular homeostasis favoring cancer development. However, while gene-specific promoter DNA hypermethylation changes are associated predominantly with deregulation of pathways