

Chapter 11

Environmental Toxicants, Epigenetics, and Cancer

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Abstract Tumorigenesis, a complex and multifactorial progressive process of transformation of normal cells into malignant cells, is characterized by the accumulation of multiple cancer-specific heritable phenotypes triggered by the mutational and/or non-mutational (i.e., epigenetic) events. Accumulating evidence suggests that environmental and occupational exposures to natural substances, as well as man-made chemical and physical agents, play a causative role in human cancer. In a broad sense, carcinogenesis may be induced through either genotoxic or non-genotoxic mechanisms; however, both genotoxic and non-genotoxic carcinogens also cause prominent epigenetic changes. This review presents current evidence of the epigenetic alterations induced by various chemical carcinogens, including arsenic, 1,3-butadine, and pharmaceutical and biological agents, and highlights the potential for epigenetic changes to serve as markers for carcinogen exposure and cancer risk assessment.

11.1 Introduction

Tumorigenesis is a complex and multifactorial progressive process of transformation of normal cells into malignant ones. It is characterized by the accumulation of multiple cancer-specific heritable phenotypes, including persistent proliferative

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

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signaling, resistance to cell death, evasion of growth suppression, replicative immortality, inflammatory response, deregulation of energy metabolism, genomic instability, induction of angiogenesis, and activation of invasion ultimately resulting in metastases [1]. The acquisition of these cancer-specific alterations may be triggered by the mutational and/or non-mutational (i.e., epigenetic) events in the genome which, in turn, affect gene expression and the downstream phenotypes listed above [1, 2]. Furthermore, it has been suggested that epigenetic alterations may play as important or even more prominent role in tumor development [3].

Epigenetic events, most prominently manifested by stable and heritable changes in gene expression that are not due to any alteration in the primary DNA sequence [4], signify the fundamental molecular principles in which genetic information is organized and read [5]. Epigenetic modifications include change in methylation patterns of cytosines in DNA [6, 7], modifications of the proteins that bind to DNA [8, 9], and the nucleosome positioning along DNA [4]. These epigenetic marks are tightly and interdependently connected and are essential for the normal development and the maintenance of cellular homeostasis and functions in adult organisms, particularly for X-chromosome inactivation in females, genomic imprinting, silencing of repetitive DNA elements, regulation of chromatin structure, and proper expression of genetic information [10]. The epigenetic status is well-balanced in normal cells, but may be altered in many ways in cancer cells. Additionally, growing evidence indicates that a number of lifestyle and environmental factors may disrupt this epigenetic balance and compromise the stability of the epigenome in normal cells leading to the development of a wide range of pathologies, including cancer.

11.2 Epigenetic Alterations in Cancer Cells

The unifying molecular feature of neoplastic cells is a profoundly reshaped genome characterized by global genomic *hypo*-methylation, gene-specific *hyper*- or *hypo*-methylation, and altered histone modification patterns [2, 11].

DNA demethylation signifies one of the two major DNA methylation states and refers to a state in which there is a decrease in the number of methylated cytosine bases from the “normal” methylation level. Demethylation of DNA can be achieved either passively or actively. Passive loss of methylated marks in the genome may be a consequence of limited availability of the universal methyl donor S-adenosyl-L-methionine (SAM), compromised integrity of DNA, and altered expression and/or activity of DNA methyltransferases [12]. Until recently, evidence for existence of an active replication-independent DNA demethylation process was controversial and inconclusive [7, 13]. However, recent studies provide compelling experimental evidence that active loss of DNA methylation is associated with the function of DNA repair machinery [14–17].

Global hypomethylation of DNA was the first epigenetic abnormality identified in cancer more than a quarter of century ago [18, 19]. It continues to be one of the

most common molecular alterations found in all human cancers [20, 21]; however, the molecular mechanisms behind cancer-linked global demethylation of the genome remain largely unknown. The loss of DNA methylation in cancer primarily affects stable, methylated areas of the genome composed predominantly of repetitive elements, genes, and intergenic regions [22].

There are several molecular consequences of global demethylation of DNA that may contribute to tumorigenesis. First, genomic hypomethylation causes significant elevation in mutation rates [23], activation of normally silenced tumor-promoting genes [24], and loss of imprinting [25]. Second, demethylation of the repetitive DNA 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, pericentromeric, and subtelomeric chromosomal regions induces their activation and transposition leading to chromosomal instability [26–29]. For example, recent findings have demonstrated that DNA hypomethylation causes permissive transcriptional activity at the centromere [28]. Subsequently, the accumulation of small minor satellite transcripts that impair centromeric architecture and function is observed. Likewise, hypomethylation of the repetitive elements at the subtelomeric regions is associated with enhanced transcription of the telomeres [29].

Gene-specific loss of DNA methylation is also a finding for oncogenes and imprinted genes. In addition, many genes that are normally well-methylated, particularly cancer-germline genes, including B melanoma antigen family (*BAGE*), cancer testis antigen (*CAGE*), melanoma antigen family A (*MAGE-A*), X antigen family (*XAGE*), and other single-copy genes, including S100 calcium binding protein A4 (*S100A4*), flap endonuclease 1 (*FEN1*), and synuclein-gamma (*SNCG*), undergo progressive hypomethylation, which is accompanied by their increased expression, in human cancers [12, 21].

Despite the large body of evidence indicating that cancer-associated DNA demethylation is an important early event in tumor development, it is still less clear if the loss of DNA methylation is a cause, or a consequence of the malignant transformation [30]. The notion that DNA hypomethylation is playing a role in causation and/or promotion of cancer is based on the results of studies with nutritional “lipogenic methyl-deficient diet” [31–33], genetically engineered *Dnmt*- and *Lsh*-deficient mice [34, 35], and several models of chemical carcinogenesis [36]. In contrast, there is also evidence that cancer-linked DNA hypomethylation may be a passive inconsequential side effect of carcinogenesis [30, 37]. The latter is evidenced by facts that not all tumors exhibit DNA hypomethylation and not all carcinogenic processes are accompanied by the loss of DNA methylation [38]. Indeed, it is highly unlikely to expect that development and progression of diverse types of tumors are all associated with DNA hypomethylation. Furthermore, there is growing evidence that DNA hypomethylation suppresses development of certain tumor types, especially intestinal, gastric, and prostate carcinomas [39–41].

DNA hypermethylation is the state where the methylation of normally under-methylated DNA domains, those that predominantly consist of CpG islands [22], increases. CpG islands are defined as the genomic regions that contain the high

G+C content, have high frequency of CpG dinucleotides, are at least 400–500 bp long, and can be located either at intragenic and intergenic, or at the 5' ends of genes [42–44]. However, only CpG islands that span 5' promoters are mainly unmethylated. For instance, less than 3% of CpG islands in gene promoters are methylated [44].

It is well-established that hypermethylation of promoter-located CpG islands causes permanent and stable transcriptional silencing of a range of protein-coding genes [45], which, along with DNA hypomethylation, plays a critical role in cancer development [2, 11]. One of the most compelling examples of the link between DNA hypermethylation and carcinogenesis is epigenetic silencing of critical tumor-suppressor genes, including cyclin-dependent kinase inhibitor 2A (*CDKN2A*; *p16^{INK4A}*), secreted frizzled-related protein (*SFRPs*) genes, adenomatous polyposis coli (*APC*), and GATA binding protein 4 (*GATA4*). The aberrant silencing of these genes allows for survival and clonal expansion of the initiated cells. Additionally, hypermethylation of several 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 leading to reduction in genomic stability and various genetic aberrations, particularly, the elevation of mutation rates in critical cancer-related genes [46, 47]. For example, the epigenetic silencing of *MGMT* leads to a greater mutation rate in *K-RAS* and *p53* genes during human colorectal carcinogenesis [48, 49]. Likewise, transcriptional inactivation of the *BRCA1* and *MLH1* genes caused by promoter hypermethylation results in elevated *p53* gene mutation frequency in human sporadic breast cancer [50] and microsatellite instability in sporadic colorectal cancer [51], respectively.

In addition to the vital role that DNA methylation state may play in the etiology and pathogenesis of cancer, it has been shown that disruption of normal patterns of covalent histone modifications is an epigenetic change frequently found in tumor cells. Histones are evolutionary conserved proteins that have globular carboxy-terminal domains critical to nucleosome formation, and flexible amino-terminal tails that protrude from the nucleosome core and contact adjacent nucleosomes to form higher order chromatin structures. At least eight different classes of post-translational modifications, including methylation, acetylation, phosphorylation, ubiquitination, sumoylation, biotinylation, and ADP-ribosylation have been identified on the core histones H2A, H2B, H3, H4, and the H1 family of linker histones [8, 9]. These histone marks are essential for organizing chromatin, maintaining genome stability, silencing repetitive DNA elements, regulating cell cycle progression, recognizing DNA damage sites and repair, and maintenance of proper expression of genetic information.

Accumulating evidence clearly indicates that cancer cells are characterized by a profoundly disturbed pattern of global and/or gene-specific histone modifications accompanied by alterations in the functioning of enzymes that are associated with those marks. There are various combinations of cancer-linked histone modifications that differ according to tumor type; however, one of the most characteristic examples of global changes in histone modifications is loss of histone H4 lysine 20

trimethylation and H4 lysine 16 acetylation, which is a common hallmark of human cancers [52].

Additionally, extensive studies in the past decade have indicated the existence and importance of another epigenetic mechanism of regulation of gene function by means of small non-coding microRNAs (miRNAs). Currently, miRNAs are recognized as one of the major regulatory gatekeepers of protein-coding genes in human genome [53, 54]. MiRNAs are small 16–29 nucleotide-long non-coding RNAs that primarily function as negative gene regulators at the post-transcriptional level [55]. MiRNAs are generated by RNA polymerase II or RNA polymerase III as long primary transcripts, primary miRNAs. Following transcription, primary miRNAs form a stem-loop structure, which is recognized and processed by the RNase III-type enzyme Droscha creating precursor miRNAs. These precursor miRNAs are transported from the nucleus to the cytoplasm by Exportin-5. In the cytoplasm, the pre-miRNAs are further processed by Dicer, an RNase III enzyme, generating miRNA:miRNA hybrids. After unwinding, one strand of the duplex is degraded, and another strand becomes a mature miRNA. MiRNAs can induce mRNA cleavage if complementary to 3'-untranslated region of targets is perfect or translational repression if complementarity is imperfect [53].

Currently there are more than 700 mammalian miRNAs that can potentially target up to one-third of protein-coding genes involved in the development, cell differentiation, metabolic regulation, signal-transduction, cell proliferation, and apoptosis. As the deregulation of these very same biological processes is a hallmark of cancer [1], it has been suggested that changes in miRNA expression might have significance in cancer [56–58]. In tumors, aberrant expression of miRNAs inhibits tumor suppressor genes or inappropriately activates oncogenes have been experimentally associated with most aspects of tumor biology, including tumor progression, invasiveness, metastasis, and acquisition of resistance of malignant cells to various chemotherapeutic agents [58]. This leads to the suggestion that altered expression of miRNAs is an important mechanism of carcinogenesis [57, 59].

11.3 Role of Epigenetic Alterations in Chemical Carcinogenesis

Many environmental and occupational exposures to natural substances, man-made chemical and physical agents are considered to be causative of human cancer [60–62]. In a broad sense, carcinogenesis may be induced through either genotoxic or non-genotoxic mechanisms. Genotoxic carcinogens are agents that interact directly or after metabolic activation with DNA, causing mutations and leading to tumor formation. Non-genotoxic carcinogens are a diverse group of chemical compounds that are known to cause tumors by mechanisms other than direct damage to DNA. The emphasis in carcinogenesis research, until recently, has focused mainly on the investigation of various molecular signaling events, DNA damage, DNA adduct repair, and genetic aberrations, despite the fact that the importance of epigenetic mechanisms in carcinogenic process was first suggested by Miller in 1970 [63].

Accumulating evidence suggests that regardless of the mechanism of action, both genotoxic and non-genotoxic carcinogens may also lead to prominent epigenetic abnormalities in tissues that are susceptible to carcinogenesis as a result of exposure [64–68]. The following sections present an overview of the epigenetic alterations induced by several carcinogens.

11.3.1 Arsenic

Arsenic is a naturally occurring element and a ubiquitous environmental contaminant which is a public health issue world-wide [69]. The major source of human exposure to arsenic is contaminated food and drinking water. Inorganic arsenic was one of the earliest identified human carcinogens [69, 70]. It is widely accepted that exposure to arsenic is associated with skin, lung, and bladder cancers [71]. Additionally, accumulating evidence indicates that long-term exposure to arsenic causes development of liver tumors [72].

Arsenic was classified as a known human carcinogen by the International Agency for Research on Cancer (IARC) in 2004, when sufficient evidence for human carcinogenicity became available [71]; even though limited evidence for animal carcinogenicity of arsenic existed. This may be explained mainly by the absence of adequate relevant animal models to study arsenic carcinogenesis. However, the experiments in transgenic mice, e.g., v-Ha-ras (Tg.AC), keratin VI/ornithine decarboxylase (K6/ODC), and p53^{+/-}, or inbred mouse strains that are prone to spontaneous cancer development provided evidence for the carcinogenicity of arsenic in animal studies. For instance, administration of arsenic to A/J mice, a strain that exhibits a susceptibility to different pulmonary pathological states including lung cancer, enhances lung tumor multiplicity and size [70, 73]. Similarly, in utero arsenic exposure of C3H/HeJ mice, which are prone to hepatocarcinogenesis, resulted in increased incidence and multiplicity of hepatocellular carcinomas in adults [74]. The most convincing evidence for the carcinogenicity of arsenic in animals has been presented in a recent report by Tokar et al. [75] that demonstrated that “whole-life” exposure of CD1 mice to arsenic causes induction of various tumors, including lung and liver.

The molecular mechanisms behind the cancer-inducing property of arsenic are not fully elucidated and remain a subject of debate. Several potential mechanisms have been proposed to explain arsenic-induced carcinogenesis, including induction of oxidative stress, DNA–protein crosslinking, chromosomal aberrations [70], disruption of signaling pathways, and epigenetic dysregulation, particularly DNA demethylation [76]. The first evidence demonstrating an association between arsenic tumorigenicity and global DNA hypomethylation was reported by Zhao et al. [77] who showed that exposure of rat liver epithelial TRL-1215 cells to arsenic in vitro led to their malignant transformation and was paralleled by global DNA demethylation. Importantly, the extent of DNA hypomethylation in the transformed cells was positively correlated with the tumorigenicity of the cells upon inoculation

into nude mice, suggesting that loss of DNA methylation may be a causative factor in arsenic-induced carcinogenesis [77]. Since then, a large amount of data has documented a substantial target organ-specific loss of global DNA methylation and repetitive element and gene-specific methylation in various in vitro and in vivo models of arsenic-induced tumorigenesis [78–80].

Several possible explanations exist for the mechanism of DNA demethylation after exposure to arsenic. First, arsenic-induced DNA hypomethylation can be explained by the absolute requirement of SAM for the biomethylation of inorganic arsenic and DNA methylation reactions [76, 81]. Therefore, the biomethylation of inorganic arsenic reduces availability of SAM for DNA and histone methylation. Second, arsenic exposure increases generation of reactive oxygen species that may cause direct damage to DNA [82, 83]. The presence of oxidative lesions in DNA (e.g., 8-oxodeoxyguanosine and 5-hydroxymethylcytosine) severely compromises the ability of DNA methyltransferases to methylate the target cytosine and leads to passive demethylation of DNA [84]. In addition, activation of DNA repair pathway promotes active demethylation of DNA [14–17]. Third, arsenic-induced oxidative stress causes depletion of the level of intracellular reduced glutathione. This consequently leads to the enhanced glutathione biosynthesis in a transsulfuration pathway, which impairs SAM biosynthesis and perturbs DNA and histone methylation reactions [85].

In addition to global and gene-specific DNA hypomethylation, arsenic exposure causes concurrent methylation-induced transcriptional silencing of a number of tumor suppressor genes, including *p53*, *CDKN2A* (*p16^{INK4A}*), Ras association domain family member 1 (*RASSF1A*), and death-associated protein kinase (*DAPK*) [73, 86, 87], various histone modification changes [88], and alterations in miRNA expression [89].

It is of note that growing evidence suggests that carcinogenesis induced by an environmental chronic exposure to other metals, such as nickel, chromium, cadmium, and mercury, may also involve molecular epigenetic alterations caused by the ability of these metals to induce damage to DNA and strongly influence intracellular molecular and metabolic alterations [90, 91].

11.3.2 1,3-Butadiene

The gaseous olefin 1,3-butadiene is a major industrial chemical monomer widely used in production of synthetic rubber, resins, and plastic. Additionally, this highly volatile agent is present in industrial and automobile exhaust, cigarette smoke, and ambient air in urban locations and industrial complexes [92]. Based on the results of numerous comprehensive epidemiological studies, the IARC has classified 1,3-butadiene as a known human carcinogen [92–94]. In rodents, it causes tumor formation at several target sites, including the hematopoietic system, lungs, heart, and liver [93]. Importantly, the hematopoietic system, lungs and liver are the most common sites of 1,3-butadiene-induced tumor formation in both humans and mice [93].

It is well-established that the mechanism of tumor induction caused by 1,3-butadiene-exposure is due to genotoxic reactivity of its metabolic epoxides: 1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane, and 3,4-epoxy-1,2-butanediol that interact directly with DNA to form mutagenic DNA adducts [94]. However, recent evidence demonstrates that short-term inhalational exposure of C57BL/6J mice to 1,3-butadiene, in addition to DNA adduct formation, also causes extensive concurrent epigenetic changes. These include a marked reduction of global DNA and repetitive element methylation and a profound loss of histone H3K9, H3K27, and H4K20 trimethylation in the livers of C57BL/6J mice [95].

It is well-established that methylation of lysine residues 9 and 27 at histone H3 and lysine 20 at histone H4 plays a fundamental role in the formation of a condensed heterochromatin structure and transcriptional repression [96–98]. Hence, loss of H3K9 and H4K20 trimethylation induced by 1,3-butadiene-exposure may compromise genomic stability via chromatin relaxation and activation of mobile repetitive elements. Indeed, a recent report showing decondensation of chromatin and activation of main repetitive elements in the livers of 1,3-butadiene-exposed C57BL/6J mice support this suggestion [99]. Additionally, an open chromatin structure may increase further vulnerability of DNA to the genotoxicity of reactive 1,3-butadiene metabolites.

The elucidation of the mechanisms of carcinogenicity is usually carried out in genetically homogeneous *in vivo* models in order to fix as many variables as possible. This provides information in a single strain, yet the extrapolation of such data to the population effects is constrained by the inference from a single genome to model complex human phenotypes. To overcome this important limitation, panels of genetically defined animals may be used to determine genetic causes of inter-individual variability in cancer susceptibility [100]. In a recent study, Koturbash et al. [99] have demonstrated substantial differences in hepatic genetic and epigenetic response among mouse strains to short-term inhalational exposure to 1,3-butadiene. More importantly, the strain differences were associated with alterations in chromatin structure, mainly in the variability in histone H3K9, H3K27, and H4K20 methylation.

11.3.3 *Pharmaceuticals*

Diethylstilbestrol is a synthetic non-steroidal estrogen that was widely used to prevent potential miscarriages and as emergency contraceptive (morning-after pill) [101]. Currently, diethylstilbestrol is classified by the IARC as a known human carcinogen [101]. Breast is the main target organ for diethylstilbestrol-induced carcinogenesis in women who were exposed during pregnancy. Additionally, diethylstilbestrol also causes development of adenocarcinoma in the uterus and cervix of women who were exposed *in utero*.

In addition to the established mechanistic genotoxic and estrogen receptor-mediated carcinogenic events, epigenetic programming also plays a substantial role.

Perinatal exposure to diethylstilbestrol causes persistent demethylation and transcriptional activation of several critical cancer-related genes in the mouse uterus, including lactoferrin (*Lf*), nucleosomal binding protein 1 (*Nsbp1*), and *c-fos* [102–104]. The mechanism of these demethylation events is associated with the ability of diethylstilbestrol to inhibit expression of the maintenance (*Dnmt1*) and de novo (*Dnmt3a* and *Dnmt3b*) DNA methyltransferases in the mouse uterus [105]. Additionally, recent evidence indicates that diethylstilbestrol exposure causes epigenetically induced down-regulation of miRNA-9 in human breast epithelial cells [106], one of the frequently down-regulated miRNAs in human breast cancer [107].

Tamoxifen, a selective non-steroidal anti-estrogen, is a widely used drug for chemotherapy and for chemoprevention of breast cancer worldwide [108]. However, recently the IARC classified tamoxifen as a known human carcinogen based on evidence for endometrial cancer [101]. One of the possible mechanisms of carcinogenic effects of tamoxifen in the uterus is tamoxifen-induced gene expression changes [109], particularly, hypomethylation-linked activation of paired box 2 (*PAX2*) gene [110].

Additionally, a number of studies have demonstrated that tamoxifen is a potent hepatocarcinogen in rats with both tumor initiating and promoting properties [111]. The mechanism of tamoxifen-induced hepatocarcinogenesis is associated with its genotoxic [112, 113] and epigenetic effects [114]. These non-genotoxic epigenetic alterations include demethylation of the entire genome and the repetitive elements, loss of global histone H4 lysine 20 trimethylation [114, 115], and altered expression of miRNAs [116]. The results of these studies further emphasize the importance of non-genotoxic mechanisms in chemical carcinogenesis induced by genotoxic carcinogens.

Phenobarbital, the most widely used anticonvulsant worldwide, is a well-established mitogenic non-genotoxic rodent liver carcinogen. It is known to increase cell proliferation, alter cell cycle checkpoint control, including delaying and attenuating the G1 checkpoint, inhibit the induction of p53, thereby resulting in accumulation of DNA damage, and induce extensive epigenetic abnormalities. Treatment with phenobarbital leads to rapid and progressive accumulation of altered DNA methylation regions in the livers of C57BL/6 and B6C3F1 mice [117]. These changes were more pronounced in livers of tumor-prone B6C3F1 and CAR (constitutive androstane receptor) wild-type mice [118]. Interestingly, the number of hypermethylated regions was noticeably smaller than hypomethylated regions, among which cytochrome P450, family 2, subfamily b, polypeptide 10 (*Cyp2b10*) gene is concomitantly hypomethylated and transcriptionally activated early after phenobarbital treatment [119].

Oxazepam is widely used as a sedative-hypnotic and antianxiety drug. Chronic exposure of B6C3F1 mice to oxazepam induces development of hepatoblastoma and hepatocellular carcinoma in mice [120]. Interestingly, oxazepam, similar to phenobarbital, causes induction of *Cyp2b10* gene in the livers of B6C3F1 mice [121, 122]. Also, oxazepam-induced tumors display a decreased expression of *Apc* and phosphatase and tensin (*Pten*) homolog tumor suppressor genes and genes involved in regulation of DNA methylation and histone modification [122].

11.3.4 Biological Agents

Mycotoxins are a structurally diverse class of molecules of fungal origin that are common contaminants of the human and animal food products [123]. Three of the most ubiquitous mycotoxins, aflatoxin B₁, fumonisin B1, and ochratoxin, are classified by the IARC as known and possible human carcinogens [124, 125]. It is well-established that aflatoxin B₁, fumonisin B1, and ochratoxin A are genotoxic carcinogens [123, 126, 127]; however, accumulating evidence indicates that their carcinogenicity involves also a complex network of epigenetic alterations [128–134].

Aflatoxin B₁ induces several epigenetic abnormalities that may induce and promote tumor development. Specifically, exposure to aflatoxin B₁ causes methylation-induced transcriptional silencing of *MGMT*, *p16^{INK4A}*, and *RASSF1A* genes, a fundamental epigenetic event in liver carcinogenesis [128–130]. Conversely, aflatoxin B₁ is a strong inducer of epigenetically regulated *SNCG* gene [131]. Additionally, a study conducted by Hu et al. [134] has demonstrated that cytosine methylation at the CpG site at codon 14 of the *K-ras* gene is the major reason for preferential aflatoxin B₁-induced DNA-adduct formation at this codon in normal human bronchial epithelial cells.

Fumonisin B₁, in addition to various genotoxic and non-genotoxic alterations, increases the level of 5-methylcytosine in genomic DNA from 5 to 9% in human intestinal Caco-2 cells [132].

Helicobacter pylori infection is associated with development of gastric cancer, one of the most prevalent human cancers worldwide [135]. The results of several comprehensive studies indicate that *H. pylori* infection causes marked DNA methylation changes in infected normal or preneoplastic gastric mucosa. *H. pylori* infection causes significant aberrant DNA methylation in a number of the promoter CpG island-containing genes, including *p16^{INK4A}*, lipoxygenase (*LOX*), heart and neural crest derivatives expressed 1 (*HAND1*), thrombomodulin (*THBD*), and actin related protein 2/3 complex, subunit p41 (*p41IARC*) gastric cancer-associated genes in gastric mucosa [136–139]. Importantly, hypermethylation of some genes, e.g., *THBD* persisted in gastric mucosa after *H. pylori* eradication [140].

11.4 Epigenetic Alterations and the Evaluation of Cancer Risk

Recognition of the fundamental role of epigenetic alterations in cancer has resulted in the identification of numerous epigenetic abnormalities that may be used as potential biomarkers for the molecular diagnosis of cancer and prognosis of survival or treatment outcomes. Despite a lack of conclusive information to clarify whether or not epigenetic changes are involved directly in neoplastic cell transformation, evidence highlighted above suggests that epigenetic alterations may be used as early indicators of carcinogenesis for both genotoxic and non-genotoxic carcinogens. Importantly, several research groups have argued that epigenetic alterations may be

used as biomarkers in the evaluation of the carcinogenic potential of the environmental factors [5, 67, 68, 141].

Incorporation of the epigenetic biomarkers into the studies on cancer risk of exposures holds a number of advantages over traditionally used methods, such as evaluation of the carcinogen-induced DNA damage, DNA adduct formation, or bacterial mutagenicity. Specifically, we reason that the following features are in favor of greater integration of epigenetic biomarkers in studies of the carcinogenic potential of the environmental exposures: (1) early appearance; (2) stability; (3) target tissue-specificity; (4) relatively low cost of the assays needed to detect these changes; (5) applicability to both genotoxic and non-genotoxic agents; and, more importantly, (6) a greater number of detectable epigenetic changes as compared to the genetic alterations after exposure.

Also, the incorporation of epigenetic technologies into the studies of cancer risk promises to enhance substantially the efficiency of carcinogenicity testing. More importantly, the reversibility of epigenetic alterations opens novel mechanism-based approaches not only to cancer treatment but also to the timely prevention of cancer [142]. However, despite a very promising outlook on the benefits of epigenetic biomarkers, additional studies are still needed to better define the nature and mechanisms of epigenetic abnormalities with respect to carcinogenic processes [60, 143, 144]. Although extensive studies have identified a number of cancer-related epigenetic abnormalities that are associated with carcinogen exposure, there is no consensus on the role of changes in tumorigenesis.

Additionally, it is possible that not all these aberrant epigenetic events are equally important for the tumorigenic process. It is highly unlikely that all of these epigenetic changes play a causative role in tumorigenesis. For example, some epigenetic changes may drive other epigenetic events that contribute to the formation of a transformed phenotype, while others may be passenger epigenetic events that accompany the transformation process [145]. In this respect, the identification of those epigenetic events that drive cell transformation is crucially important for understanding mechanisms of tumorigenesis and for cancer prevention.

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