Published in final edited form as:
Adv Exp Med Biol. 2013 ; 754: 215–232. doi:10.1007/978-1-4419-9967-2_11.

Environmental toxicants, epigenetics, and cancer

Igor P. Pogribny[1] and **Ivan Rusyn**[2]

[1]Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079, USA

[2]Department of Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC 27599, USA

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.

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 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

Corresponding authors: Igor P. Pogribny, M.D., Ph.D., Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079. Phone: (870) 543-7096; Fax: (870) 543-7720 igor.pogribny@fda.hhs.gov. Ivan Rusyn, M.D., Ph.D., Department of Environmental Sciences & Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, Phone/Fax: (919) 843-2596, iir@unc.edu.

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

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.

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 intracesternal 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 undermethylated 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 genes (*SFRPs*), 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, ubiquitynation, 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 Drosha 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].

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.

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/ornitine 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].

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 andH4K20 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] has 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

3.3. Pharmaceuticals

Diethylstilbestrol is a synthetic nonsteroidal 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 microRNA-9 in human breast epithelial cells [106], one of the frequently down-regulated microRNAs in human breast cancer [107].

Tamoxifen, a selective nonsteroidal 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 microRNAs [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 homolog (*Pten*) tumor suppressor genes and genes involved in regulation of DNA methylation and histone modification [122].

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 B_1, fumonisin B1, and ochratoxin, are classified by the IARC as known and possible human carcinogens [124,125]. It is well-established that aflatoxin B_1, 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 B_1 induces several epigenetic abnormalities that may induce and promote tumor development. Specifically, exposure to aflatoxin B_1 causes methylation-induced transcriptional silencing of *MGMT*, *p16^{INK4A}*, and *RASSF1A* genes, a fundamental epigenetic event in liver carcinogenesis [128-130]. Conversely, aflatoxin B_1 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 B_1-induced DNA-adduct formation at this codon in normal human bronchial epithelial cells.

Fumonisin B_1, in addition to various genotoxic and nongenotoxic alterations, increases the level of 5-methylcytosine in genomic DNA from 5% to 9% in human intestinal Caco-2 cells [132].

Helicobacter pylori (H. 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 (*p41ARC*) 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].

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: (i) early appearance; (ii) stability; (iii) target tissue-specificity; (iv) relatively low cost of the assays needed to detect these changes, (v) applicability to both genotoxic and non-genotoxic agents, and, more importantly, (vi) 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 in 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 [145]. 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 [146]. 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.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer. Cell. 2011; 144:646–674. [PubMed: 21376230]
2. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007; 128:683–692. [PubMed: 17320506]
3. Ushijima T, Asada K. Aberrant DNA methylation in contrast with mutations. Cancer Sci. 2010; 101:300–305. [PubMed: 19958364]

4. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010; 31:27–36. [PubMed: 19752007]
5. Marlowe J, Teo SS, Chibout SD, Pognan F, Moggs JJ. Mapping the epigenome – impact for toxicology. EXS. 2009; 99:259–288. [PubMed: 19157065]
6. Kim JK, Samaranayake M, Pradhan S. Epigenetic mechanisms in mammals. Cell Mol Life Sci. 2009; 66:596–612. [PubMed: 18985277]
7. Ooi SK, O'Donnell AH, Bestor TH. Mammalian cytosine methylation at a glance. J Cell Sci. 2009; 122:2787–2791. [PubMed: 19657014]
8. Chen ZX, Riggs AD. DNA methylation and demethylation in mammals. J Biol Chem. 2011; 286:18347–18353. [PubMed: 21454628]
9. Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–1080. [PubMed: 11498575]
10. Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]
11. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358:1148–1159. [PubMed: 18337604]
12. Pogribny IP, Beland FA. DNA hypomethylation in the origin and pathogenesis of human diseases. Cell Mol Life Sci. 2009; 66:2249–2261. [PubMed: 19326048]
13. Ooi SK, Bestor TH. The colorful history of active DNA demethylation. Cell. 2008; 133:1145–1148. [PubMed: 18585349]
14. Ma DK, Guo JU, Ming GL, Song H. DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. Cell Cycle. 2009; 8:1526–1531. [PubMed: 19377292]
15. He YF, Li BZ, Li Z, Wang Y, Tang Q, Ding J, Jiz Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, Xu GL. Tet-mediated formation of 5-hydroxylcytosine and its excision by TDG in mammalian DNA. Science. 2011; 333:1303–1307. [PubMed: 21817016]
16. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011; 333:1300–1333. [PubMed: 21778364]
17. Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, Le Coz M, Devarajan K, Wessels A, Soprano D, Abramowitz LK, Bartolomei MS, Rambow F, Bassi MR, Bruno T, Fanciulli M, Renner C, Klein-Szanto AJ, Matsumoto Y, Kobi D, Davidson I, Alberti C, Larue L, Bellacosa A. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell. 2011; 146:67–79. [PubMed: 21722948]
18. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004; 4:143–153. [PubMed: 14732866]
19. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics. 2009; 1:239–259. [PubMed: 20495664]
20. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. Biochim Biophys Acta. 2007; 1775:138–162. [PubMed: 17045745]
21. De Smet C, Loriot A. DNA hypomethylation in cancer: Epigenetic scars of a neoplastic journey. Epigenetics. 2010; 5:206–213. [PubMed: 20305381]
22. Rollins RA, Haghighi F, Edwards JR, Das R, Zhang MQ, Ju J, Bestor TH. Large-scale structure of genomic methylation patterns. Genome Res. 2006; 16:157–163. [PubMed: 16365381]
23. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature. 1998; 395:89–93. [PubMed: 9738504]
24. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene. 2002; 21:5400–5413. [PubMed: 12154403]
25. Feinberg AP, Cui H, Ohlsson R. DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms. Semin Cancer Biol. 2002; 12:389–398. [PubMed: 12191638]
26. Howard G, Eiges R, Gaudet F, Jaenisch R, Eden A. Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. Oncogene. 2008; 27:404–408. [PubMed: 17621273]
27. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003; 300:455. [PubMed: 12702868]

28. Wong NC, Wong LH, Quach JM, Canham P, Craig JM, Song IZ, Clark SJ, Choo KH. Permissive transcriptional activity at the centromere through pockets of DNA hypomethylation. PLoS Genet. 2006; 2:e17. [PubMed: 16477312]
29. Vera E, Canela A, Fraga MF, Esteller M, Blasco MA. Epigenetic regulation of telomeres in human cancer. Oncogene. 2008; 27:6817–6833. [PubMed: 18762811]
30. Wild L, Flanagan JM. Genome-wide hypomethylation in cancer may be a passive consequence of transformation. Biochim Biophys Acta. 2010; 1806:50–57. [PubMed: 20398739]
31. Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer Res. 1992; 52:2071s–2077s. [PubMed: 1544143]
32. Christman JK. Dietary effects on DNA methylation: do they account for hepatocarcinogenic properties of lipotrope diets? Adv Exp Med Biol. 1995; 369:141–154. [PubMed: 7541179]
33. Pogribny IP, James SJ, Jernigan S, Pogribna M. Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. Mutat Res. 2004; 548:53–59. [PubMed: 15063136]
34. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. Induction of tumors in mice by genomic hypomethylation. Science. 2003; 300:489–492. [PubMed: 12702876]
35. Fan T, Schmidtmann A, Xi S, Briones V, Zhu H, Suh HC, Gooya J, Keller JR, Xu H, Roayaei J, Anver M, Ruscetti S, Muegge K. DNA hypomethylation caused by Lsh deletion promotes erythroleukemia development. Epigenetics. 2008; 3:134–142. [PubMed: 18487951]
36. Counts JL, Goodman JI. Hypomethylation of DNA: an epigenetic mechanism involved in tumor promotion. Mol Carcinog. 1994; 11:185–188. [PubMed: 7999259]
37. Wild L, Funes JM, Boshoff C, Flanagan JM. In vitro transformation of mesenchymal stem cells induces gradual genomic hypomethylation. Carcinogenesis. 2010; 31:1854–1862. [PubMed: 20466760]
38. Bagnyukova TV, Tryndyak VP, Montgomery B, Churchwell MI, Karpf AR, James SR, Muskhelishvili L, Beland FA. Genetic and epigenetic changes in rat preneoplastic liver tissue induced by 2-acetylaminofluorene. Carcinogenesis. 2008; 29:638–646. [PubMed: 18204080]
39. Yamada Y, Jackson-Grusby L, Linhart H, Meissner A, Eden A, Lin H, Jaenish R. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. Proc Natl Acad Sci USA. 2005; 102:13580–13585. [PubMed: 16174748]
40. Tomita H, Hirata A, Yamada Y, Hata K, Oyama T, Mori H, Yamashita S, Ushijima T, Hara A. Suppressive effect of global DNA hypomethylation on gastric carcinogenesis. Carcinogenesis. 2010; 31:1627–1633. [PubMed: 20622006]
41. Kinney SR, Moser MT, Pascual M, Greally JM, Foster BA, Karpf AR. Opposing roles of Dnmt1 in early- and late-stage murine prostate cancer. Mol Cell Biol. 2010; 30:4159–4174. [PubMed: 20584988]
42. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJM, Haussler D, Marra MA, Hirst M, Wang T, Costello JF. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010; 466:253–257. [PubMed: 20613842]
43. Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. Proc Natl Acad Sci USA. 2002; 99:3740–3745. [PubMed: 11891299]
44. Illingworth RS, Bird AP. CpG islands – "a rough guide". FEBS Lett. 2009; 583:1713–1720. [PubMed: 19376112]
45. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011; 25:1010–1022. [PubMed: 21576262]
46. Jacinto FV, Esteller M. Mutator pathways unleashed by epigenetic silencing in human cancer. Mutagenesis. 2007; 22:247–253. [PubMed: 17412712]
47. Lahtz C, Pfeifer GP. Epigenetic changes of DNA repair genes in cancer. J Mol Cell Biol. 2011; 3:51–58. [PubMed: 21278452]
48. Esteller M, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Watkins DN, Issa JP, Sidransky D, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-

DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. Cancer Res. 2000; 60:2368–2371. [PubMed: 10811111]

49. Esteller M, Riques RA, Toyota M, Capella G, Moreno V, Peinado MA, Baylin SB, Herman JG. Promoter hypermethylation of the DNA repair gene O6-methylguanine-DNA methyltransferase is associated with the presence G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. Cancer Res. 2001; 61:4689–4692. [PubMed: 11406538]
50. Birgisdottir V, Stefansson OA, Bodvarsdottir SK, Hilmarsdottir H, Jonasson JG, Eyfjorf JE. Epigenetic silencing and deletion of the BRCA1 gene in sporadic breast cancer. Breast Cancer Res. 2006; 8:R38. [PubMed: 16846527]
51. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA. 1998; 95:6870–6875. [PubMed: 9618505]
52. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Lyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet. 2005; 37:391–400. [PubMed: 15765097]
53. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–233. [PubMed: 19167326]
54. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009; 136:642–655. [PubMed: 19239886]
55. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010; 466:835–840. [PubMed: 20703300]
56. Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annu Rev Med. 2009; 60:167–179. [PubMed: 19630570]
57. Ventura A, Jacks ST. MicroRNAs and cancer: short RNAs go a long way. Cell. 2009; 136:586–591. [PubMed: 19239879]
58. Di Leva G, Croce CM. Roles of small RNAs in tumor formation. Trends Mol Med. 2010; 16:257–267. [PubMed: 20493775]
59. Pogribny IP. MicroRNA dysregulation during chemical carcinogenesis. Epigenomics. 2009; 1:281–290. [PubMed: 22122703]
60. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. Cancer Res. 2008; 68:6863–6872. [PubMed: 18757397]
61. Wild CP. Environmental exposure measurement in cancer epidemiology. Mutagenesis. 2009; 24:117–125. [PubMed: 19033256]
62. Clapp RW, Jacobs MM, Loechler EL. Environmental and occupational causes of cancer: new evidence 2005-2007. Rev Environ Health. 2008; 23:1–37. [PubMed: 18557596]
63. Miller JA. Carcinogenesis by chemicals: and overview – G.H.A. Clowes memorial lecture. Cancer Res. 1970; 30:559–576. [PubMed: 4915745]
64. Herceg Z. Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagenesis. 2007; 22:91–103. [PubMed: 17284773]
65. Pogribny IP, Rusyn I, Beland FA. Epigenetics aspects of genotoxic and non-genotoxic hepatocarcinogenesis: studies in rodents. Environ Mol Mutagen. 2008; 49:9–15. [PubMed: 17879298]
66. Bollati V, Baccareli A. Environmental epigenetics. Heredity. 2010; 105:105–112. [PubMed: 20179736]
67. Nakajima T, Enomoto S, Ushijima T. DNA methylation: a marker for carcinogen exposure and cancer risk. Environ Health Prev Med. 2008; 13:8–15. [PubMed: 19568874]
68. Ziech D, Franco R, Pappa A, Malamou-Mitsi V, Georgakila S, Georgakitas AG, Panayiotidis MI. The role of epigenetics in environmental and occupational carcinogenesis. Chem Biol Interact. 2010; 188:340–349. [PubMed: 20599843]
69. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: a historical perspective. Toxicol Sci. 2011; 123:305–332. [PubMed: 21750349]

70. Rossman TG. Mechanism of arsenic carcinogenesis: an integrated approach. Mutat Res. 2003; 533:37–65. [PubMed: 14643412]
71. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. Some drinking-water disinfectants and contaminants, including arsenic. Vol. 84. IARC; Lyon, France: 2004.
72. Liu J, Waalkes MP. Liver is a target of arsenic carcinogenesis. Toxicol Sci. 2008; 105:24–32. [PubMed: 18566022]
73. Cui X, Wakai T, Shirai Y, Hatakeyama K, Hirano S. Chronic oral exposure to inorganic arsenate interferes with methylation status of p16INK4A and RASSF1A and induces lung cancer in A/J mice. Toxicol Sci. 2006; 91:372–381. [PubMed: 16543296]
74. Waalkes MP, Ward JM, Liu J, Diwan BA. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol. 2003; 186:7–17. [PubMed: 12583988]
75. Tokar EJ, Diwan BA, Ward JM, Delker DA, Waalkes MP. Carcinogenic effects of “whole-life” exposure to inorganic arsenic in CD1 mice. Toxicol Sci. 2011; 119:73–83. [PubMed: 20937726]
76. Reichard JF, Puga A. Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. Epigenomics. 2010; 2:87–104. [PubMed: 20514360]
77. Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes MP. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proc Natl Acad Sci USA. 1997; 94:10907–10912. [PubMed: 9380733]
78. Chen H, Li S, Liu J, Diwan BA, Barrett JC, Waalkes MP. Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis. Carcinogenesis. 2004; 25(9):1779–1786. [PubMed: 15073043]
79. Jensen TJ, Novak P, Elbin KE, Gandolfi AJ, Futscher BW. Epigenetic remodeling during-arsenical-induced malignant transformation. Carcinogenesis. 2008; 29:1500–1508. [PubMed: 18448484]
80. Ren X, McHale CM, Skibola CF, Smith AH, Smith MT, Zhang L. An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis. Environ Health Perspective. 2011; 119:11–19.
81. Coppin JF, Qu W, Waalkes MP. Interplay between cellular methyl metabolism and adaptive efflux during oncogenic transformation from chronic arsenic exposure in human cells. J Biol Chem. 2008; 283:19342–19350. [PubMed: 18487201]
82. Kitchin KT, Ahmad S. Oxidative stress as a possible mode of action for arsenic carcinogenesis. Toxicol Lett. 2003; 137:3–13. [PubMed: 12505428]
83. Kojima C, Ramirez DC, Tokar EJ, Himeno S, Drobná Z, Stýblo M, Mason RP, Waalkes MP. Requirement of arsenic biomethylation for oxidative DNA damage. J Natl Cancer Inst. 2009; 101:1670–1681. [PubMed: 19933942]
84. Valinluck V, Sowers LC. Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. Cancer Res. 2007; 67:946–950. [PubMed: 17283125]
85. Lee DH, Jacobs DR Jr, Porta M. Hypothesis: a unifying mechanism for nutrition and chemicals as lifelong modulators of DNA hypomethylation. Environ Health Perspect. 2009; 117:1799–1802. [PubMed: 20049195]
86. Mass MJ, Wang L. Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. Mutat Res. 1997; 386:263–277. [PubMed: 9219564]
87. Huang YC, Huang WC, Chen WT, Yu HS, Chai CY. Sodium arsenite-induced DAPK hypermethylation and autophagy via ERK1/2 phosphorylation in human uroepithelial cells. Chem Biol Interact. 2009; 181:254–262. [PubMed: 19577553]
88. Zhou X, Sun H, Ellen TP, Chen H, Costa M. Arsenite alters global histone H3 methylation. Carcinogenesis. 2008; 29:1831–1836. [PubMed: 18321869]
89. Beezhold K, Liu J, Kan H, Meighan T, Castranova V, Shi X, Chen F. miR-190-mediated downregulationof PHLP contributes to arsenic-induced Akt activation and carcinogenesis. Toxicol Sci. 2011 in press.

90. Salnikow K, Zhitkovich A. Genetic and epigenetic mechanisms in metals carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. Chem Res Toxicol. 2008; 21:28–44. [PubMed: 17970581]

91. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics. 2011:820–827. [PubMed: 21610324]

92. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. 1,3-Butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride, and vinyl bromide). Vol. 97. IARC; Lyon, France: 2008.

93. Melnick RL, Sills RC. Comparative carcinogenicity of 1,3-butadiene, isoprene and chloroprene in rats and mice. Chem Biol Interact 136. 2001; 136:27–42.

94. Walker VE, Walker DM, Meng Q, McDonald JD, Scott BR, Selikop SK, Claffey DJ, Upton PB, Powley MW, Swenberg JA, Henderson RF, Health Review Committee. Genotoxicity of 1,3-butadiene and its epoxy intermediate. Res Rep Health Eff Inst. 2009; 144:3–79. [PubMed: 20017413]

95. Koturbash I, Scherhag A, Sorrentino J, Sexton K, Bodnar W, Tryndyak V, Latendresse JR, Swenberg JA, Beland FA, Pogribny IP, Rusyn I. Epigenetic alterations in liver of C57BL/6J mice after short-term inhalational exposure to 1,3-butadiene. Environ Health Perspect. 2011; 119:635–640. [PubMed: 21147608]

96. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D, Jenuwein T. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev. 2004; 18:1251–1262. [PubMed: 15145825]

97. Martens JH, O'Sullivan RJ, Braunschweig U, Opravil S, Radolf M, Steinlein P, Jenuwein T. The profile of repeat-associated histone lysine methylation states in the mouse epigenome. EMBO J. 2005; 24:800–812. [PubMed: 15678104]

98. Jenuwein Y. The epigenetic magic of histone lysine methylation. FEBS J. 2006; 273:3121–3135. [PubMed: 16857008]

99. Koturbash I, Scherhag A, Sorrentino J, Sexton K, Bodnar W, Swenberg JA, Beland FA, Pardo-Manuel Devillena F, Rusyn I, Pogribny IP. Epigenetic mechanisms of mouse interstrain variability in genotoxicity of the environmental toxicant 1,3-butadiene. Toxicol Sci. 2011; 122:448–456. [PubMed: 21602187]

100. Rusyn I, Gatti DM, Wilshire T, Kleeberger SR, Threadgill DW. Toxicogenomics: population-based testing of drug and chemical safety in mouse models. Pharmacogenomics. 2010; 11:1127–1136. [PubMed: 20704464]

101. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. A review of human carcinogens. Part A: Pharmaceuticals. Vol. 100. IARC; Lyon, France: 2011.

102. Li S, Washburn KA, Moore R, Uno T, Teng C, Newbold RR, McLachlan JA, Negishi M. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. Cancer Res. 1997; 57:4356–4359. [PubMed: 9331098]

103. Li S, Hansman R, Newbold R, Davis B, McLachlan JA, Barrett JC. Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. Mol Carcinog. 2003; 38:78–84. [PubMed: 14502647]

104. Tang WY, Newbold R, Mardilovich K, Jefferson W, Cheng RY, Medvedovic M, Ho SM. Persistent hypomethylation in the promoter of nucleosomal binding protein 1 (Nsbp1) with overexpression of Nsbp1 in mouse uteri neonatally exposed to diethylstilbestrol or genistein. Endocrinology. 2008; 149:5922–5931. [PubMed: 18669593]

105. Sato K, Fukata H, Kogo Y, Ohgane J, Shiota K, Mori C. Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in the mouse uterus. Endocr J. 2009; 56:131–139. [PubMed: 18997445]

106. Hsu PY, Detherage DE, Rodriguez BA, Liyanarachchi S, Weng YI, Zuo T, Liu J, Cheng AS, Huang TH. Xenoestrogen-induced epigenetic repression of microRNA-9-3 in breast epithelial cells. Cancer Res. 2009; 69:5936–5945. [PubMed: 19549897]

107. Lehmann U, Hasemeier B, Christgen M, Müller M, Römermann D, Länger F, Kreipe H. Epigenetic inactivation of microRNA gene has-mir-9-1 in human breast cancer. J Pathol. 2008; 214:17–24. [PubMed: 17948228]

108. Jordan VC. Tamoxifen (ICI146,474) as a target therapy to treat and prevent breast cancer. Br J Pharmacol. 2006; 147:S269–S276. [PubMed: 16402113]
109. Shang Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. Nat Rev Cancer. 2006; 6:360–368. [PubMed: 16633364]
110. Wu H, Chen Y, Liang J, Shi B, Wu G, Zhang Y, Wang D, Li R, Yi X, Zhang H, Sun L, Shang Y. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature. 2005; 438:981–987. [PubMed: 16355216]
111. Wogan GN. Review of the toxicology of tamoxifen. Semin Oncol. 1997; 24:S87–S97.
112. Phillips DH. Understanding the genotoxicity of tamoxifen? Carcinogenesis. 2001; 22:839–849. [PubMed: 11375888]
113. Gamboa da Costa G, McDaniel-Hamilton LP, Heflich RH, Margues MM, Beland FA. DNA adduct formation and mutant induction in Sprague-Dawley rats treated with tamoxifen and its derivatives. Carcinogenesis. 2001; 22:130701315.
114. Tryndyak VP, Muskhelishvili L, Kovalchuk O, Rodriguez-Juarez R, Churchwell MI, Ross SA, Beland FA, Pogribny IP. Effect of long-term tamoxifen exposure on genotoxic and epigenetic changes in rat liver: implications for tamoxifen-induced hepatocarcinogenesis. Carcinogenesis. 2006; 27:1713–1720. [PubMed: 16632870]
115. Tryndyak VP, Kovalchuk O, Muskhelishvili L, Montgomery B, Rodriguez-Juarez R, Melnyk S, Ross SA, Beland FA, Pogribny IP. Epigenetic reprogramming of liver cells in tamoxifen-induced rat hepatocarcinogenesis. Mol Carcinog. 2007; 46:187–197. [PubMed: 17219426]
116. Pogribny IP, Tryndyak VP, Boyko A, Rodriguez-Juarez R, Beland FA, Kovalchuk O. Induction of microRNAome deregulation in rat liver by long-term tamoxifen exposure. Mutat Res. 2007; 619:30–37. [PubMed: 17343880]
117. Bachman AN, Phillips JM, Goodman JI. Phenobarbital induces progressive patterns of GC-rich and gene-specific altered DNA methylation in the liver of tumor-prone B6C3F1 mice. Toxicol Sci. 2006; 91:393–405. [PubMed: 16537655]
118. Phillips JM, Goodman JI. Multiple genes exhibit Phenobarbital-induced constitutive active/androstane receptor-mediated DNA methylation changes during liver tumorigenesis and in liver tumors. Toxicol Sci. 2009; 108:273–289. [PubMed: 19233941]
119. Lempiäinen H, Müller A, Brasa S, Teo SS, Roloff TC, Morawiec L, Zamurovic N, Vicart A, Funhoff E, Couttet P, Schübeler D, Grenet O, Marlowe J, Moggs J, Terranova R. Phenobarbital mediates an epigenetic switch at the constitutive androstane receptor (CAR) target gene Cyp2b10 in the liver of B6C3F1 mice. PLoS One. 2011; 6:e18216. [PubMed: 21455306]
120. Bucher JR, Shackelford CC, Haseman JK, Johnson JD, Kurtz PJ, Persing RL. Carcinogenicity studies of oxazepam in mice. Fundam Appl Toxicol. 1994; 23:280–297. [PubMed: 7982536]
121. Iida M, Anna CH, Hartis J, Bruno M, Wetmore B, Dubin JR, Sieber S, Bennett L, Cunningham ML, Paules RS, Tomer KB, Houle CD, Merrick AB, Sills RC, Devereux TR. Changes in global gene and protein expression during early mouse liver carcinogenesis induced by non-genotoxic model carcinogens oxazepam and Wyeth-14,643. Carcinogenesis. 2003; 24:757–770. [PubMed: 12727805]
122. Lahousse SA, Hoenerhoff M, Collins J, Ton TV, Massinde T, Olson D, Rebolosso Y, Koujitani T, Tomer KB, Hong HH, Bucher J, Sills RC. Gene expression and mutation assessment provide clues of genetic and epigenetic mechanisms in liver tumors of oxazepam-exposed mice. Vet Pathol. 2011; 48:678–699.
123. Ferguson LR, Philpott M. Nutrition and mutagenesis. Annu Rev Nutr. 2008; 28:313–329. [PubMed: 18399774]
124. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol. 82. IARC; Lyon, France: 2002.
125. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010; 31:71–82. [PubMed: 19875698]
126. Knasmüller S, Bresgen N, Kassie F, Mersch-Sundermann V, Gelderblom W, Zöhrer E, Eckl PM. Genotoxic effects of three Fusarium mycotoxins, fumonisin B1, moniliformin and vomitoxin in bacteria and primary cultures of rat hepatocytes. Mutat Res. 1997; 391:39–48. [PubMed: 9219547]

127. Wang JS, Groopman JD. DNA damage by mycotoxins. Mutat Res. 1999; 424:167–181. [PubMed: 10064859]
128. Zhang YJ, Ahsan H, Chen Y, Lunn RM, Wang LY, Chen SY, Lee PH, Chen CJ, Santella RM. High frequency of promoter hypermethylation of RASSF1A and p16 and its relationship to aflatoxin B1-DNA adduct levels in human hepatocellular carcinoma. Mol Carcinog. 2002; 35:85–92. [PubMed: 12325038]
129. Zhang YJ, Chen Y, Ahsan H, Lunn RM, Chen SY, Santella RM. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation and its relationship to aflatoxin B1-DNA adducts and the p53 mutations in hepatocellular carcinoma. Int J Cancer. 2003; 103:440–444. [PubMed: 12478658]
130. Su H, Zhao J, Xiong Y, Xu T, Zhou F, Yuan Y, Zhang Y, Zhuang SM. Large-scale analysis of the genetic and epigenetic alterations in hepatocellular carcinoma from Southeast China. Mutat Res. 2008; 641:27–35. [PubMed: 18358501]
131. Zhao W, Liu H, Liu W, Wu Y, Chen W, Jiang B, Zhou Y, Xue R, Luo C, Wang L, Jiang JD, Liu J. Abnormal activation of the synuclein-gamma gene in hepatocellular carcinomas by epigenetic alteration. Int J Oncology. 2006; 28:1081–1088.
132. Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE. Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. Toxicon. 2007; 49:306–317. [PubMed: 17109910]
133. Marin-Kuan M, Cavin C, Delatour T, Schilter B. Ochratoxin A carcinogenicity involves a complex network of epigenetic mechanisms. Toxicon. 2008; 52:195–202. [PubMed: 18649906]
134. Hu W, Feng Z, Tang M. Preferential carcinogen-DNA adduct formation at codons 12 and 14 in the human K-ras gene and their possible mechanisms. Biochemistry. 2003; 42:10012–10023. [PubMed: 12924950]
135. IARC Monographs on the Evaluations of Carcinogenic Risks to Humans. A review of human carcinogens. Part B: Biological agents. Vol. 100. IARC; Lyon, France: 2011.
136. Maekita, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, Sugimura T, Ichinose M, Ushijima T. Clin Cancer Res. 2006; 12:989–995. [PubMed: 16467114]
137. Nakajima T, Yamashita S, Maekita T, Niwa T, Nakazawa K, Ushijima T. The presence of methylation fingerprint of Helicobacter pylori infection in human gastric mucosae. Int J Cancer. 2009; 124:905–910. [PubMed: 19035455]
138. Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. Inflammatory processes triggered by Helicobacter pylori infection cause aberrant DNA methylation in gastric epithelial cells. Cancer Res. 2010; 70:1430–1440. [PubMed: 20124475]
139. Shin CM, Kim N, Jung Y, Park JH, Kang GH, Kim JS, Jung HC, Song IS. Role of Helicobacter pylori infection in aberrant DNA methylation along multistep gastric carcinogenesis. Cancer Sci. 2010; 101:1337–1346. [PubMed: 20345486]
140. Nakajima T, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, Oda I, Gotoba T, Ushijima T. Persistence of a component of DNA methylation in gastric mucosa after Helicobacter pylori eradication. J Gastroenterol. 2010; 45:37–44. [PubMed: 19821005]
141. LeBaron MJ, Rasoulpour RJ, Klapacz J, Ellis-Hutchings RG, Hollnagel HM, Gollapudi BB. Epigenetics and chemical safety assessment. Mutat Res. 2010; 705:83–95. [PubMed: 20399890]
142. Huang YW, Kuo CT, Stoner K, Huang TH, Wang LS. An overview of epigenetics and chemoprevention. FEBS Lett. 2011; 585:2129–2136. [PubMed: 21056563]
143. Goodman JI, Augustine KA, Cunningham ML, Dixon D, Dragan YP, Falls JG, Rasoulpour RJ, Sills RC, Storer RD, Wolf DC, Pettit SD. What do we need to know prior to thinking about incorporating an epigenetic evaluation into safety assessments? Toxicol Sci. 2010; 116:375–381. [PubMed: 20430866]
144. Rasoulpour RJ, LeBaron MJ, Ellis-Hutchings RG, Klapacz J, Gpllapudi BB. Epigenetic screening in product safety assessment: are we there yet? Toxicol Mech Methods. 2011; 21:298–311. [PubMed: 21495868]

145. Kalari S, Pfeifer GP. Identification of driver and passenger DNA methylation in cancer by epigenomic analysis. Adv Genet. 2010; 70:277–308. [PubMed: 20920752]