Chapter 32

Chemical Carcinogens and Their Effect on Genome and Epigenome Stability

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Chapter Outline

1. INTRODUCTION

Cancer is a disease that is characterized by the uncontrolled growth, proliferation, and spread of cells. This deadly disease is projected to affect one in four Americans and Canadians. Development of cancer (the process of carcinogenesis) is a long, multistep transformation of normal cells into malignant cells, which includes initiation, promotion, and progression stages from single initiated cells to carcinoma in situ and further to large-scale carcinoma and metastasis [1–3]. For a long time, cancer was thought to be a genetic disease, whereby pathological changes were caused by a progressive accumulation of a multitude of genetic and cytogenetic alterations. Since the first cancer-promoting mutation in a human *RAS* oncogene was reported in 1983, a large body of evidence has accumulated on various polymorphisms, point mutations, deletions, insertions, and translocations associated with cancer development [2].

Induction and development of some cancers can be genetically predetermined via inherited mutations that are passed from generation to generation, such as those in BRCA1, MSH2, MLH1, and other genes, albeit those direct gene defects account only for 5–10% of cases.

A large proportion of cancers is induced by the harmful influence of deleterious cancer-causing factors—carcinogens that can cause mutations or alter the proper function and stability of the cellular genome, leading to a loss of cellular growth controls. Carcinogens can be of a chemical, physical (UV radiation, X-rays, and magnetic fields), or biological (bacterial, viral, or altered metabolism) nature. Chemical carcinogens account for a lion's share of all induced cancers and can be divided into genotoxic and non-genotoxic ones based on their mechanisms of action and, especially, based on their capability to alter the DNA sequence [4]. Historically, the term *genotoxic carcinogen* has been used to define a chemical that is "capable of producing cancer by directly altering the genetic material of target cells." A *non-genotoxic carcinogen* refers to "a chemical capable of producing cancer by some secondary mechanism not related to direct gene damage" [5].

As such, most genotoxic carcinogens interact with DNA or produce metabolites that can react with DNA, and these direct reactions with DNA alter DNA and the chromosome structure or chromosome number. Some genotoxic carcinogens (eg, ethyl methane sulfate) act directly, while others require metabolic activation (2-acetylaminofluorene). Genotoxic carcinogens cause various types of DNA damage, including base alkylation, oxidation, base loss, formation of DNA adducts, interstrand cross-links, DNA–protein cross-links, and breaks in DNA. As a main repository of genetic information, DNA is the only cellular molecule that is repaired; the rest of them are simply replaced.

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To combat these attacks on the genome, cells have evolved a response system that induces cell-cycle arrest, allowing sufficient time for specialized groups of proteins to repair the incurred damage. To protect themselves, cells harbor elaborate and highly effective DNA-repair machinery that includes more than 100 proteins and a precise cell cycle–control system that induces cell-cycle arrest to allow time for repairs. Consequently, in the vast majority of cases, DNA damage is repaired. Moreover, in the case of irreparable damage, the cellular DNA damage–response system induces apoptosis. If the damage is unrepaired or misrepaired, this can lead to mutation and result in the phenomenon of genome instability that manifests as an elevated accumulation of mutations that are persistent in the cellular lineage. Genomic instability can facilitate the process of cancer initiation and/or progression [6], and indeed, the loss of genomic stability is believed to be a hallmark of many cancers, as well as an important prerequisite for cancer formation [7–9].

Non-genotoxic carcinogens do not affect DNA directly, but rather cause epigenetic changes, affect gene expression, and thus cause metabolic changes, increase peroxisome proliferation, disrupt cellular structures, change the rate of cell proliferation, or foster other processes that are responsible for cellular homeostasis. Disruption of those processes may in turn predispose cells to indirect DNA damage and can lead to carcinogenesis.

Yet, even though numerous spontaneous and carcinogen-induced cancer-causing mutations have been identified and cataloged, they cannot explain, by far, a wide variety of different malignant tumors (cancers and soft tissue tumors) and their relationship with environmental factors or some of the puzzling patterns of tumor predisposition and inheritance. Additionally, numerous lines of evidence have suggested that cancer can arise due to aberrant gene expression and regulation [2]. As such, it is now well accepted that cancer is both a genetic and an epigenetic disease [1,2]. Additionally, research conducted in 2009 indicates that genetic and epigenetic mechanisms can mediate the toxicity of various environmental chemicals, both genotoxic and non-genotoxic ones [4].

2. EPIGENETIC REGULATORS

Modern science defines *epigenetics* as mechanisms that establish and maintain mitotically and meiotically stable and heritable patterns of gene expression and regulation and occur without changes in DNA sequence. Epigenetic processes impact gene expression and chromatin structure and include DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs [2,10].

2.1 DNA Methylation

Among epigenetic regulators, cytosine DNA methylation was the first epigenetic mark identified, and it is one of the most widely studied epigenetic phenomena. DNA methylation is a covalent modification of DNA, in which a methyl group from S-adenosyl-L-methionine is added to the carbon 5 position of cytosine, yielding 5-methylcytosine (5 mC) in DNA. Genomic DNA methylation refers to the overall content of methylated cytosine (5 mC) in the genome [2,11].

DNA methylation is a key regulator of gene expression and genome stability. It is crucial for the proper functioning of normal cells and tissues. In normal cells, it governs the regulation of cell-type and tissue-specific gene expression, the silencing of parasitic and highly repetitive sequences, X-chromosome inactivation, the correct organization of active and inactive chromatin, and genomic imprinting [10,12] (Fig 32.1). In mammals, including humans, DNA methylation occurs mainly in the context of CpG dinucleotides that are methylated to 70–90% [11]. The highest frequency of CpGs is in the CpG island areas, which are often located in the 5′-end control regions of genes [11,12].

DNA methylation is accomplished by DNA methyltransferase enzymes [11]. In mammals, three DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) are responsible for establishing and maintaining DNA-methylation patterns at CpG sites [10]. DNMT1 is the major enzyme involved in the maintenance of DNA-methylation patterns after DNA replication. It is localized at replication forks where, in collaboration with de novo methyltransferases and methyl-binding proteins, it directly modifies nascent DNA strands after replication and thereby maintains DNA-methylation patterns. DNMT3a and DNMT3b are de novo methyltransferases that target unmethylated and hemimethylated sites and that initiate and establish DNA methylation. Deregulation of methyltransferases may lead to altered methylation patterns [11]. DNA methylation is known to be associated with the inactive chromatin state and, in most cases, with repressed gene expression activity, while the loss of DNA methylation oftentimes correlates with elevated gene expression.

The altered patterns of genomic DNA methylation constitute a well-known characteristic of cancer cells [1,2,11]. Both hypermethylation and hypomethylation alterations occur in cancer (Fig. 32.1). The DNA-methylation profile of cancer cells is characterized by global genome DNA hypomethylation, cancer-associated gene-specific hypomethylation, and concurrent hypermethylation of CpG islands within the gene promoters of tumor suppressors [1,2,11,12].

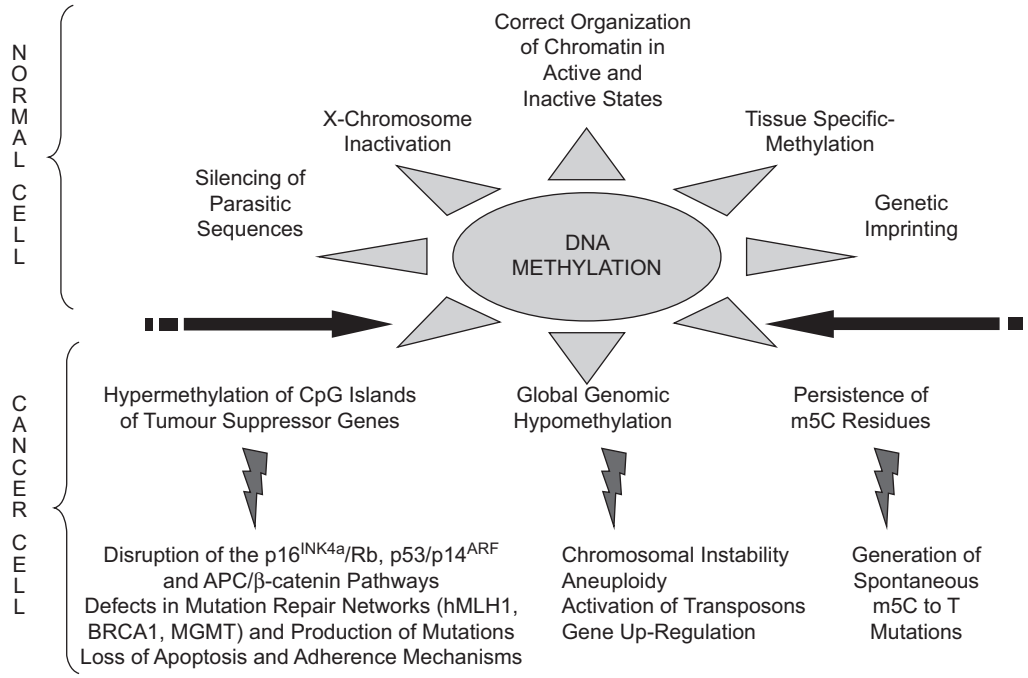

FIGURE 32.1 DNA methylation at the center of the normal and malignant behavior of the cell. *Adopted with permission from Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. J Pathol 2002;196(1):1–7.*

The global loss of genomic DNA methylation has been linked to the activation of transposable elements, such as long and short interspersed nucleotide elements (LINEs and SINEs), retroviral intracisternal (A) particles (IAPs), and other elements located in the centromeric, pericentromeric, and subtelomeric chromosomal regions. It is also associated with elevated levels of chromosome breakage, aneuploidy, and increased mutation rates. All of these are signs of global genomic instability and the hallmarks of carcinogenesis [11].

Alongside global genomic DNA hypomethylation, cancer cells exhibit gene-specific hypomethylation. This is one of the prominent and potent mechanisms for the reactivation of oncogenes. Up to now, several hypomethylated tumor-promoting genes have been identified in major cancers. These include proto-oncogenes, plasminogen activators, urokinase (UPA), heparanase (HPA), and many others [1,2,11,12].

Hypermethylation, the gain of methylation at sites that are normally undermethylated, is another characteristic feature of cancer cells. Hypermethylation is the most extensively studied epigenetic change in cancer. Aberrant promoter methylation leads to silencing of a large number of protein-coding genes as well as genes coding for small RNAs [11]. Genes affected by DNA hypermethylation include crucial tumor-suppressor genes, such as the key gatekeeper p53 and retinoblastoma, cyclin-dependent kinase inhibitor 2A (p16INK4A), adenomatous polyposis coli (APC) gene, and Ras association (RalGDS/AF-6) domain family member 1 (RASSF1A), just to name a few. Hypermethylation may also affect DNA-repair genes, such as breast cancer 1 and 2 genes (BRCA1 and BRCA2), MutL homolog 1 (MLH1), and genes involved in apoptosis control [2,11].

In sum, in cancer, aberrant DNA methylation causes altered gene expression and regulation and leads to the deregulation of key processes that are critical for tumor initiation and progression, such as cell growth and persistent proliferative signaling, replicative immortality, resistance to apoptosis, signal transduction, inflammation, angiogenesis, and invasion [1,2,11,12] (Fig. 32.1).

2.2 Histone Modifications

The basic structure that comprises the chromatin is the nucleosome or beads on a string. It is essentially comprised of a strand of DNA being wrapped around an octamer of histone proteins containing a tetramer of H3–H4 histone proteins with an H2A–H2B dimer situated on either side. These four proteins are classified as core histones. A fifth histone, H1 (or linker histone), secures this structure and is involved in higher-order chromatin packing. These proteins are made up of a high proportion of positively charged amino acids, such as lysine and arginine, which allows them to interact electrostatically

with net negatively charged DNA. The interactions between the nucleic acids and histone proteins in this structure function to organize and pack DNA and can have important implications for gene expression. Furthermore, histone proteins are often subjected to modifications, particularly on their N-terminal tails, which may alter their interactions with the associated DNA strand and contribute to the differential regulation of gene expression. Included in these alterations are acetylation, deacetylation, methylation, phosphorylation, and ubiquitination [13,14].

2.3 RNA-Induced Effects

This field was first initiated in the 1990s with the discovery of transgene silencing, first observed in *Petunia hybrida* in 1990 [15]. At first, the mechanism behind this phenomenon was unknown, but it was determined upon a later study that it was actually the transgene-derived RNA mediating the sequence-specific silencing. RNA-induced silencing was first seen in animals by Fire and Mello (1998) using *Caenorhabditis elegans*. Since these initial findings, the knowledge base surrounding RNA-mediated regulation of gene expression has expanded to include several groups of small RNA, including microRNA (miRNA), small interfering RNA (siRNA), and piwi-interacting RNA (piRNA) [16]. All are distinguished by their small size (ranging from 20 to 30 nucleotides), their role as a guiding agent for members of the Argonaute (Ago) family of proteins, and their involvement in reducing the expression of target genes [16]. Among those, miRNAs are key major negative regulators of expression of the vast majority of mammalian protein-coding genes [17]. miRNAs are key players in carcinogenesis and important biomarkers of cancer predisposition, development, and treatment outcomes [18–21].

Numerous environmental factors have been linked to aberrant changes in epigenetic pathways, both in experimental and epidemiological studies. In addition, epigenetic mechanisms may mediate specific mechanisms of toxicity and responses to certain chemicals [4].

3. EFFECTS OF METALS

Several studies have established an association between DNA methylation, and environmental metals and metalloids, including nickel, chromium, lead, cadmium, and particularly arsenic. Metals can cause oxidative stress because of the elevated production of reactive oxygen species (ROS) via redox signaling. Oxidative DNA damage has been shown to alter the activity of DNA methyltransferases and to affect their ability to interact with DNA, thus leading to aberrant DNA-methylation patterns.

Among toxic metals, cadmium is associated with several cancers, such as lung, kidney, uterine, and ovarian cancer [22–25], albeit it has very low direct mutagenic potential [26]. Cadmium exposure stems from tobacco smoke, air pollution, and diet, as well as from some occupational exposures [27]. Mechanistically, cadmium exposure has been shown to cause elevated ROS and reduced global genome DNA methylation via the noncompetitive inhibition of DNA methyltransferases [4,28,29]. Along with decreased global DNA methylation, cadmium also leads to hypomethylation and the aberrant expression of proto-oncogenes, thus inducing cellular proliferation and transformation [29]. Cadmium exposure has been shown to cause hypermethylation and decreased expression of the tumor-suppressor genes *RASSF1A* and *P16*, along with the overexpression and increased activity of DNMT activity [30,31]. Cadmium exposures cause aneuploidy that may be mediated by global DNA hypermethylation, which may be one of the mechanisms of cadmium-induced carcinogenesis [32]. Along with hypermethylation, cadmium can cause DNA hypomethylation of LINE1-transposable elements. Loss of LINE1 methylation is a common epigenetic event in malignancies and may also be important for cadmium-induced carcinogenesis [33].

Nickel, one of the most abundant metals, is found in coins, jewelry, stainless steel, batteries, and medical devices. Occupational nickel exposures occur during refinery, plating, and welding operations, and have been significantly associated with liver, lung, nasal, and pharyngeal cancers [34], although the precise mechanisms of nickel carcinogenicity are largely unknown [25]. Nickel exposures induce DNA damage, oxidative stress, and epigenetic alterations [35,36]. Studies during 1995 and 1998 showed that nickel exposure leads to promoter hypermethylation and increased global DNA methylation [37,38]. Nickel also causes significant posttranslational histone modification effects [27,39,40]. Nickel exposures has also been shown to cause hypermethylation and repression of several tumor-suppressor and DNA-repair genes, such as O6-methylguanine DNA methyltransferase, *MGMT*, *RARβ2*, *RASSF1A*, and the *P16* promoter [41,42].

Over the past few decades, environmental lead exposures have significantly decreased due to regulations banning or decreasing the use of lead in gasoline and paint [27,43], although the general population is still exposed due to tobacco smoke [44]. Lead exposures increase the risk of cardiovascular, kidney, and neurocognitive diseases and cancer [27]. Lead exposure has been reported to cause changes in DNA methylation and global gene expression, specifically, global hypomethylation [45–47], but the precise mechanisms of lead-induced changes in DNA methylation need to be further delineated.

Hexavalent chromium (Cr (VI)) is another well-known human and animal carcinogen [48]. People are environmentally exposed to chromium by drinking chromium-contaminated water or by using chromium-containing products, such as dyes, paints, inks, and plastics. Occupational chromium exposure has been associated with the elevated incidence of lung, stomach, liver, and kidney cancer [49–52]. Even though Cr (VI) is a known mutagen, it exerts its carcinogenicity largely through epigenetic mechanisms [25]. Several previous studies have highlighted the potential epigenetic effects of chromium (Cr(VI)), especially DNA-methylation effects [53]. Chromate Cr (VI) exposure has been associated with the hypermethylation of certain genes involved in the cell cycle control and DNA repair, such as *MLH1*, *p16*, *and APC* genes [54–56].

Arsenic is a common metalloid element and a human carcinogen [25]. Arsenic exposure exerts acute and chronic toxicity and has been associated with skin, lung, liver, and bladder cancers, as well as cardiovascular and neurological diseases [57–59]. Altered DNA methylation is a known sign of arsenic-induced carcinogenesis, and arsenic has been shown to cause both DNA hypermethylation and hypomethylation. Several studies have associated arsenic with gene-specific hypermethylation, whereby arsenic causes hypermethylation of the *P53* and *P16* genes, as well as the death-associated protein kinase (*DAPK*) gene. Additionally, arsenic causes an increase in global 5mC, indicative of global DNA hypermethylation [60]. Along with hypermethylation, global DNA hypomethylation is an early event in some cancers and occurs in response to arsenic exposure [61]. Moreover, arsenic exposures also affect other epigenetic mechanisms, such as histone modifications.

Overall, epigenetic changes play intricate roles in the regulation of gene expression upon metal exposures. The unique methylome alterations have been displayed in cancer cells after exposure to carcinogenic metals, such as nickel, lead, arsenic, cadmium, and chromium (VI). The metal-stimulated deviations to the methylome are possible mechanisms for metal-induced carcinogenesis and may provide potential biomarkers for cancer detection. These mechanisms are discussed in depth in an elegant review by Brocato and Costa (2013) [25].

Moreover, in-depth systematic analyses carried out in 2015 established that miRNA changes are caused by exposures to toxic chemicals and may be sensitive biomarkers of toxicant exposure [62,63]. Also, miRNAs are important players in arsenic-induced carcinogenesis. Luo et al., in 2014, demonstrated that arsenic-induced malignant transformation of lung HBE cells is associated with an increased expression of oncogenic miR-21 [64]. This miRNA has been found to be overexpressed in virtually all human cancers and is implicated in the carcinogenic process through the regulation of cell proliferation, genome instability, inflammation, evading apoptosis, invasion and metastasis, and angiogenesis [63]. Arsenic exposure can lead to upregulated miR-190 and result in downregulation of the PH domain leucine-rich repeat protein phosphatase (PHLPP) through the direct interaction of miR-190 with the 3′-UTR of the PHLPP mRNA, leading to activation of the AKT-signaling pathway [65].

Similarly to HBE cells, miR-21 can be upregulated by arsenic in immortalized human keratinocytes [66]. Additionally, arsenic exposure can lead to the reduction of let-7a, let-7b, let7c, and miR-34a during malignant transformation [63,67]. In arsenic-induced bladder carcinogenesis, arsenic treatment has resulted in the downregulation of the anti-EMT miRNAs, miR-200a, miR-200b, and miR-200c. In transformed human prostate epithelial cells, arsenic exposure has been shown to cause the downregulation of miR-134, miR-138, miR-155, miR-181c, miR-181d, miR-205, miR-373, and let-7 [68]. Decreased expression of the aforementioned miRNAs has been associated with the overexpression of target genes, RAN, RAB22A, RAB27A, and KRAS, which are key regulators of carcinogenesis. Additionally, arsenic exposure can cause the pronounced upregulation of miR-9, a small RNA that targets the miRNA-processing enzyme DICER1 [69].

Nickel exposure causes dose-dependent elevated miRNA-21 expression and promotes lung tumorigenesis [70]. Furthermore, the level of miR-152 can be significantly downregulated in nickel-transformed cells compared to passage-matched control cells. Additionally, in experimental nickel-induced carcinogenesis, miR-222 was upregulated, and miR-203 was downregulated [71–73].

Chromium exposure causes decreased levels of miR-143. He and colleagues suggested that the downregulation of miR-143 promotes chromium-induced cell transformation through increasing the expression of the insulin-like growth factor-1 receptor and the insulin receptor substrate-1 and through further activation of the ERK/hypoxia-induced factor 1α/NF-κB-signaling pathway [74].

In comparison to arsenic, little is known about the role of miRNA deregulation in cadmium-induced carcinogenesis. An analysis made in 2015 and 2016 showed that cadmium exposure results in the significant deregulation of multiple miRNAs that are predicted to target cell-cycle regulation, p53-signaling, and Wnt-signaling pathways [75,76].

4. TAMOXIFEN EFFECTS

Tamoxifen is a selective nonsteroidal anti-estrogen that has been used in the treatment of breast cancer since mid-1980s. It has been used lately as an effective chemopreventive agent for breast cancer in women who have a high risk of developing breast cancer [77,78]. While tamoxifen has proven to be beneficial for preventing the occurrence or recurrence of breast

cancer [79], the IARC has classified it as a known human carcinogen, since it has been shown to increase the incidence of endometrial cancer [80,81]. Additionally, tamoxifen is a potent hepatocarcinogen in rodents, where it exhibits both cancer-initiating and cancer-promoting properties [82–85].

Mechanistically, tamoxifen-induced hepatic tumors in rats occur, at least in part, due to a genotoxic mechanism resulting from the formation of tamoxifen–DNA adducts [86–90]. Along with genotoxic mechanisms, tamoxifen causes profound gene expression and epigenetic changes [91–94].

High-throughput microarray technology has allowed researchers to establish the gene expression profiles in liver tissues during the early stages of tamoxifen-induced rat hepatocarcinogenesis. Global gene expression profiling of the liver tissues of rats treated with tamoxifen for 12 or 24 weeks indicated that the early stages of tamoxifen-induced liver carcinogenesis are characterized by alterations in several major cellular pathways, such as those involved in drug metabolism, lipid metabolism, cell cycle, apoptosis, and cell-proliferation control. Tamoxifen exposure can cause significant, progressive, and sustained increases in expression of the Pdgfc, Calb3, Ets1, and Ccnd1 genes, accompanied by an elevated level of the PI3K, p-PI3K, Akt1/2, Akt3, and cyclin B, D1, and D3 proteins [93].

An analysis of tamoxifen-induced epigenetic changes revealed pronounced global genomic DNA demethylation and altered activity and expression maintenance DNA methyltransferase (DNMT1) and de novo (DNMT3a and DNMT3b) DNA methyltransferases. Tamoxifen-induced DNA hypomethylation was paralleled by the progressive loss of histone H4K20me3 in liver tissues of tamoxifen-treated animals [92] and the loss of global histone H4 lysine 20 trimethylation [92]. Tamoxifen exposure also caused accumulation of DNA lesions in the liver tissues of tamoxifen-treated female F344 rats. Moreover, long-term exposure of female F344 rats to tamoxifen led to a substantial and progressive loss of CpG methylation in the regulatory sequences of LINE-1 and a subsequent pronounced increase in the levels of expression of LINE-1 elements and the c-myc proto-oncogene, that are known manifestations of genome instability. The accumulation of tamoxifen-induced changes was accompanied by the decreased level of key DNA-repair proteins, Rad51, Ku70, and DNA polymerase beta, which are very important for the maintenance of genome stability. Molecular and epigenetic changes were paralleled by increased regenerative cell proliferation, and taken together, the data showed that exposure of animals to tamoxifen led to the emergence of cancer-related epigenetic phenotypes prior to tumor formation [91].

In another study, the treatment of Fisher 344 rats to tamoxifen for 24 weeks caused substantial changes in the expression of miRNA genes in the liver. In this study, tamoxifen exposure caused a significant upregulation of known oncogenic miRNAs, such as the 17–92 cluster, miR-106a, and miR-34, and miRNA changes resulted in corresponding changes in the expression of proteins targeted by these miRNAs, including cell-cycle regulators, chromatin modifiers, and the expression regulators implicated in the regulation of genome stability and carcinogenesis. Moreover, the observed tamoxifen-induced miRNA changes occur prior to tumor formation and are not merely a consequence of a transformed state [94].

5. EFFECTS OF 1,3-BUTADIENE

Environmental contamination by numerous industrial chemicals is becoming a serious global problem. The gaseous olefin 1,3-butadiene is one such industrial chemical that is widely used in the production of plastic, rubber, and resins. This highly volatile chemical constitutes a key component of industrial and automobile exhaust and is also found in cigarette smoke. Furthermore, it is commonly found in urban ambient air and industrial complexes [95]. The International Agency for Research on Cancer (IARC) has classified 1,3-butadiene as a known human and rodent carcinogen that is associated with lung, liver, and hematopoietic system cancers [95–97].

1,3-Butadiene is a well-known genotoxic carcinogen, and the main mechanism of tumor induction by 1,3-butadiene exposure is the formation of its highly reactive metabolic epoxides (1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane, and 3,4-epoxy-1,2-butanediol), which cause the formation of DNA adducts [96]. While its genotoxic potential has been established, this potential does not fully explain all of the carcinogenic mechanisms of 1,3-butadiene effects. Studies during 2011 and 2014 by the Pogribny and Rusyn laboratories have established that 1,3-butadiene profoundly alters gene expression and epigenetic processes in the affected cells and tissues [98–100].

In 2011, in a series of elegant studies, murine exposure to 1,3-butadiene also resulted in profound changes in global gene expression patterns, causing altered expression of Hmox1, Nqo1, Car3, Srebf1, and Lgr5, all of which are indicative of liver injury. The short-term inhalation of 1,3-butadiene in C57BL/6J mice caused, along with DNA adduct formation, extensive epigenetic changes such as a significant decrease in global DNA methylation, marked hypomethylation of repetitive elements, and a pronounced loss of histone H3K9, H3K27, and H4K20 trimethylation that are signs of genome instability in the liver tissue [99].

The methylation of lysine residues 9 and 27 at histone H3 and lysine 20 at histone H4 is crucially important for heterochromatin formation and maintenance as well as for transcriptional repression and general stability of the genome.

Therefore, the observed 1,3-butadiene exposure-induced loss of H3K9 and H4K20 trimethylation may in turn contribute to chromatin relaxation and overall genome instability. Indeed, a 2011 report further proved the decondensation of chromatin and activation of repetitive elements in the livers of 1,3-butadiene-exposed C57BL/6J mice [98,99].

Such an open chromatin structure may in turn increase the vulnerability of DNA to the influence of genotoxic DNA-reactive metabolites of 1,3-butadiene and contribute to adduct formation. Analysis also revealed significant interstrain differences in genetic and epigenetic responses to the inhalational of 1,3-butadiene in murine liver tissues, and these strain differences are associated with differences in histone H3K9, H3K27, and H4K20 methylation levels and alterations in chromatin structure [98].

Furthermore, epigenetic changes may underlie the tissue specificity of 1,3-butadiene-induced genome instability and tumorigeneses. A 2014 study by the Rusyn Laboratory analyzed 1,3-butadiene-induced changes in the kidney, liver, and lung tissues of mice that had had inhalational exposure. They noted that while 1,3-butadiene exposure caused DNA damage in all three tissues, epigenetic changes varied between the kidney, liver, and lung tissues of the exposed animals. 1,3-Butadiene-induced epigenetic changes indicative of genome instability included demethylation of repetitive DNA sequences and alterations in histone–lysine acetylation levels observed in the liver and lung tissues of the exposed mice. On the other hand, no DNA-methylation changes were seen in the kidneys of the exposed mice. Moreover, the histone marks of condensed heterochromatin and transcriptional silencing (histone–lysine trimethylation) were increased in kidney tissue, suggesting genome-stabilization effects. Therefore, epigenetic in-tissue differences may help to explain the differences in cancer predisposition. These modifications may represent a potential mechanistic explanation for the tissue specificity of cancer predisposition upon exposure to 1,3-butadiene [100]. As of 2016, no studies have analyzed the effects of 1,3-butadiene exposure on tissue microRNAome, and such analysis may provide more insight into the mechanisms of 1,3-butadiene-induced carcinogenesis.

6. INFLUENCE OF POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are the most widespread organic pollutants found in the environment. They are extensively present in crude oil, coal, and tar deposits and originate from the burning of fossil fuels and forest fires. Other significant PAH exposures stem from automobile exhaust, cigarette smoke, industrial exposure at coal-tar production plants, and municipal trash incinerators [101]. PAHs also come from dietary fats and overused cooking oils [102,103]. Human exposure to PAHs is associated with a wide array of diseases and conditions such as asthma, obstructive lung disease, heart disease, as well as lung, bladder, and other cancers [101,103]. PAH exposure significantly affects the in utero development of children, having been associated with cognitive defects and fetal growth impairment. One of the most sensitive periods for PAH exposure is during early-embryonic development. A large-scale longitudinal cohort study involving 700 children revealed significant epigenetic effects from transplacental PAH exposure [104]. Further studies have shown that maternal PAH exposure leads to aberrant global DNA methylation and gene-specific methylation, as well as the accumulation of DNA adducts in cord blood [103,105].

PAH exposures reportedly cause formation of DNA adducts, and they have also been associated with increased methylation levels of *Alu* and *LINE-1* and abnormal DNA-methylation patterns at specific sequences of the *p53* gene promoter, which correlate with the levels of PAH exposure seen in chronically exposed industrial coke–oven workers [106]. In occupationally exposed firefighters, PAH exposure has led to a higher level of *DUSP22* promoter hypomethylation in blood DNA, when compared with unexposed controls [107].

The carcinogenic activity of PAHs has often been associated with the induction of genotoxic and non-genotoxic alterations that both lead to genome instability [63]. Among those, exposure to the PAH benzo[*a*]pyrene has led to very pronounced formation of *anti*-7β, 8α-dihydroxy-9α, 10α-epoxy-7, 8, 9,10-tetrahydrobenzo[*a*]pyrene adducts. Interestingly, these occurred at the major hot-spot mutation codons 157, 248, and 273 of the *P53* gene and at codon 14 in the human *KRAS* gene—key genes implicated in a wide array of cancers [108,109]. Along with adducts, PAH benzo[*a*]pyrene exposures have also been shown to cause alterations in global and gene-specific DNA-methylation status and to affect genome stability in cancer-targeted tissues and lines [103,110].

PAHs, and especially PAH benzo[*a*]pyrene (BP) exposure, have been shown to cause changes in the expression of several miRNAs that may contribute to genome instability and malignant cell transformation. To this effect, a study by Shen and colleagues showed that benzo[*a*]pyrene exposure caused neoplastic transformation of normal human bronchial epithelial cells, which they associated with the profound upregulation of 45 and the downregulation of nine miRNAs [111]. Also upregulated were oncogenic miRNAs such as miR-17-5p, miR-20a, miR-92, miR-106a, miR-129, miR-320, miR-494, and miR498. Conversely, miR-10a, miR-363*, and miR-493-5p were significantly downregulated [111].

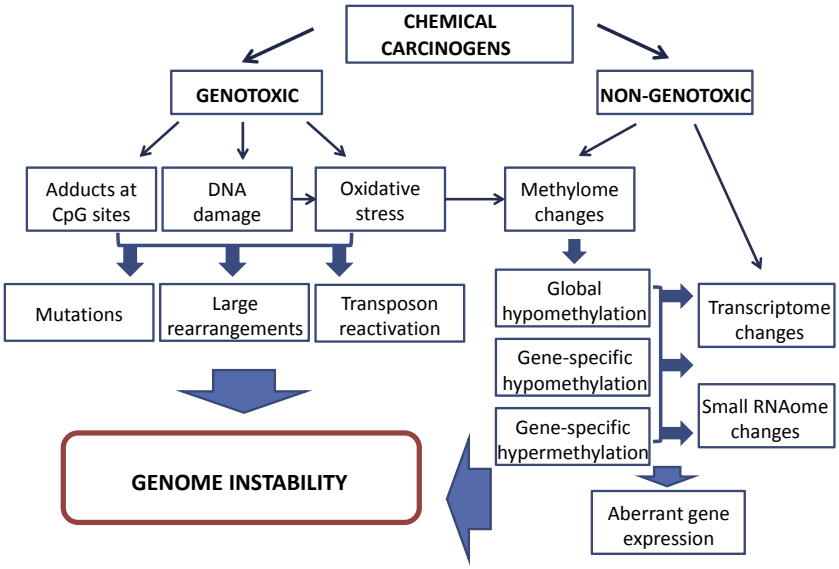

FIGURE 32.2 Genetic and epigenetic effects of carcinogens on genome stability. Exposure to genotoxic chemical carcinogens causes direct or indirect DNA damage, as well as effects on methylome, and subsequent aberrant global gene expression. Additionally, genotoxic effects on the methylome can lead to the increased levels of carcinogen–DNA-adduct formation at methylated CpG sites. Unrepaired or misrepaired damage can lead to genome instability. Non-genotoxic carcinogens affect methylome, cause oxidative stress, and cause global loss of DNA methylation, gene-specific hypermethylation, and gene-specific hypomethylation. Moreover, DNA hypomethylation can also lead to elevated mutation rates and genome instability. *Modified after Pogribny IP, Beland FA. DNA methylome alterations in chemical carcinogenesis. Cancer Lett 2013;334(1):39–45.*

BP-induced upregulation of miR-17-92 and miR-106a was important in the BP-induced malignant transformation of HBE cells. Increasing miR-106a levels in the transformed cells caused increased proliferation and inhibited apoptosis. Contrarily, inhibition of oncogenic miR-106a in the BP-transformed cells inhibited cell proliferation, induced cell arrest and apoptosis, and also inhibited growth of tumor xenografts in nude mice [112].

Several other studies reported BP-induced upregulation of miR-22, miR-494, and miR-638 and downregulation of miR-10a, miR-34c, and miR-506 during BP-induced carcinogenesis.

MicroRNAs were also deregulated in peripheral lymphocytes of PAH-exposed workers; among those, upregulation occurred in miR-638 [113] and other miRNAs that target tumor-suppressor proteins such as miR-20a, miR-17-5p, miR-106a, miR-494, miR-22, and miR-34c.

Several miRNAs were downregulated by BP exposure; among those, miR-10a was one of the most downregulated miRNAs in BP-transformed human 16HBE cells and in the lungs of rats exposed to BP-containing tobacco smoke [111,114].

7. CONCLUSIONS

Numerous environmental toxicants and chemicals have been reported to cause both genetic and epigenetic effects that play a key role in chemical-induced genome instability and carcinogenesis. These include endocrine disruptors—important environmental xenobiotics that interfere with the normal development and functioning of male and female reproductive systems and cause numerous health effects. The most studied endocrine disruptors include vinclozolin, methoxychlor, and other pesticides, as well as plasticiser bisphenol A, all of which act at different levels of epigenetic control and cause transgenerational effects [115–119].

Mycotoxins are toxic compounds of fungal origin that are common contaminants in human and animal food products. Among these, aflatoxin B_1, fumonisin B1, and ochratoxin are known to be possible human carcinogens [120,121]. While they are genotoxic carcinogens, they have also been reported to cause a complex network of epigenetic alterations [122–124]. Both genetic and epigenetic mechanisms of toxicity and carcinogenicity were described for alcohol exposure, cigarette smoke, chemotherapy agents, and many other toxicants. Many of these were described in several elegant review articles published in 2015 [63,125–131].

In sum, epigenetic modifications are influenced by the environment and environmental toxicants, including chemical carcinogens. The majority of genotoxic and non-genotoxic carcinogens affect genome stability and cause cancer by perturbing epigenetic processes in the cells (Fig. 32.2). These carcinogen-induced epigenetic changes are stable and can be used as important exposure biomarkers. On the other hand, epigenetic changes are pliable and reversible, and, therefore, analysis of chemical carcinogens-induced epigenetic alterations may uncover novel mechanism-based approaches for cancer treatment and cancer prevention. Furthermore, in the future, the incorporation of epigenetic technologies in carcinogenesis analysis and cancer-risk assessment will enhance the efficiency of carcinogenicity testing.

GLOSSARY

Genotoxic carcinogen A chemical that is capable of producing cancer by directly altering the genetic material of target cells.
Non-genotoxic carcinogen A chemical capable of producing cancer by some secondary mechanism not related to direct gene damage.

LIST OF ABBREVIATIONS

IAPs Intracisternal (A) particles
LINEs Long interspersed nucleotide elements
miRNA MicroRNA
PAHs Polycyclic aromatic hydrocarbons
piRNA piwi-interacting RNA
SINEs Short interspersed nucleotide elements
siRNA Small interfering RNA

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