Published in final edited form as:

Crit Rev Toxicol. 2013 July ; 43(6): . doi:10.3109/10408444.2013.794769.

Basic Mechanics of DNA Methylation and the Unique Landscape of the DNA Methylome in Metal-Induced Carcinogenesis

Jason Brocato and **Max Costa***

Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, NY 10987, USA

Abstract

DNA methylation plays an intricate role in the regulation of gene expression and events that compromise the integrity of the methylome may potentially contribute to disease development. DNA methylation is a reversible and regulatory modification that elicits a cascade of events leading to chromatin condensation and gene silencing. In general, normal cells are characterized by gene-specific hypomethylation and global hypermethylation, while cancer cells portray a reverse profile to this norm. The unique methylome displayed in cancer cells is induced after exposure to carcinogenic metals such as nickel, arsenic, cadmium, and chromium (VI). These metals alter the DNA methylation profile by provoking both hyper- and hypomethylation events. The metal-stimulated deviations to the methylome are possible mechanisms for metal-induced carcinogenesis and may provide potential biomarkers for cancer detection. Development of therapies based on the cancer methylome requires further research including human studies that supply results with larger impact and higher human relevance.

Keywords

DNA methylation; metals; hypomethylation; hypermethylation; promoter methylation; nickel; arsenic; chromium; cadmium; cancer; methylome; metal carcinogenesis; 5mC; DNMT; tet proteins

I. Introduction

All of our cells contain the same DNA. So how does a hepatocyte function differently from a keratinocyte? Different genes are turned on and off in each cell type allowing each cell to perform specific functions that other cells cannot. One way a cell can turn on and off genes is through DNA methylation. DNA methylation is an epigenetic mark that labels the chromatin on (open state) or off (closed state) (Ooi et al., 2009). DNA methylation occurs in both prokaryotes and eukaryotes. In prokaryotes, the DNA is methylated on adenine and it functions to protect the host's DNA from digestion by restriction enzymes that are designed to eliminate foreign DNA (Noyer-Weidner and Trautner, 1993). In eukaryotes, DNA

*Corresponding Author Tel. (845)-371-3552, Max.Costa@nyumc.org.

Declaration of Interest

The employment affiliation of the authors is as shown on the cover page. This paper was prepared during the normal course of the authors' employment. The paper was prepared as part of the graduate program for Jason Brocato who is supported by Environmental Health Sciences. Other support for preparation of the paper was provided by grants to Max Costa from NIH grant numbers ES000260, ES010344, ES014454, ES005512, 5 T32 ES007324 from the National Institutes of Environmental Health Sciences, and support from grant number CA16087 from National Cancer Institute. The paper was written exclusively by the authors and the views and opinions expressed in the paper are exclusively those of the authors.

methylation occurs primarily on cytosines in CpG base pairs (CGs) and its main function is to regulate gene expression (Miranda and Jones, 2007).

This review will discuss the formation, localization, and downstream effects of DNA methylation. We will describe the altered methylation state of cancer cells and how exposure to carcinogenic metal compounds containing nickel, arsenic, cadmium, and chromium can alter the cell's DNA methylation profile to resemble the methylome of a cancer cell. A guide with all abbreviations and genes used in this article can be found in supplementary table 1.

Methyl modifications on DNA act as regulatory marks that can up-regulate or down-regulate gene expression depending on the genomic location and density of the marks (Miranda and Jones, 2007). In the first section of this review, we will discuss where these epigenetic marks occur throughout the genome in mammalian cells and their influence on transcription. The enzymes and proteins that create (writers), recognize (readers), and remove (erasers) methylated cytosines will be reviewed to gain an understanding of the dynamics of the modification. Because cancer cells have a unique DNA methylation profile, the next section of the review will describe the DNA methylation alterations seen in cancer due to both hyper- and hypomethylation events. Metal-induced carcinogenesis alters the cell's DNA methylation profile to resemble the methylome of a cancer cell. We will describe sources of exposure and discuss human, animal and *in vitro* investigations characterizing metal compounds containing nickel, arsenic, cadmium, and hexavalent chromium and discuss specific instances where these metals induce cancer-associated hyper- and hypomethylation events.

II. Genomic Locations and Functions of DNA Methylation

A. Occurrence of DNA methylation throughout the genome

Methylation of cytosine forms 5-methylcytosine (5mC) and in mammals this modification most often occurs on cytosines in CGs; however, in stem cells 25% of 5mC occurs next to adenine (Ramsahoye et al., 2000). Throughout the mammalian genome, CGs may occur in clusters or as isolated sites. Clusters of CGs, which are CG rich sequences, often occur in the promoter regions of genes and are called CpG islands (CGIs) (Fazzari and Grealley, 2004). In mammalian cells, CGIs are usually unmethylated and are usually found within promoters of transcriptionally active genes, such as housekeeping genes and tissue-specific genes. Upstream of CGIs, there may be sequences less dense in CGs termed CG shores. Shores are usually found flanking CGIs and can be methylated or unmethylated. CGs that do not occur in CG-rich regions are isolated CGs and may be found in repetitive sequences, transposable elements, non-repetitive intergenic DNA, and exons of genes. These isolated CGs are usually methylated and are associated with transcriptionally inactive regions of chromatin (Choudhuri et al., 2010).

B. Influence of DNA methylation on gene expression

Methylation of the CGIs in promoters of genes is associated with gene silencing. The hypermethylated promoter can down-regulate gene expression by interfering with the binding of transcription factors (TFs) and recruiting methylated DNA-binding proteins (MBPs) (Choudhuri et al., 2010). In normal cells, promoter regions are protected from methylation by binding TFs such as Sp1 which render them inaccessible to DNA methyltransferases (DNMTs). When the promoter is methylated, TFs are prevented from binding and transcription is not activated (Brandeis et al., 1994). MBPs can facilitate silencing by recruiting corepressor complexes that lead to condensed chromatin and this subject will be discussed later in the review.

Another role of DNA methylation is its control over the activation of transposable elements (TEs). TEs are DNA sequences that can change their relative position (self-transpose) within the genome of a single cell and their activation can result in genomic instability. Methylation of TEs silences these sequences and prevents them from mobilizing to other genomic locations (Yagi et al., 2012). When CGs in TEs lose their methylation, the TEs become mobilized and insert into genomic locations such as regulatory sequences and gene bodies and may disrupt gene expression. Differential methylation of TEs located within or near promoters of genes can also interfere with normal gene expression (Dolinoy et al., 2006, Yagi et al., 2012).

III. Writers, Readers, and Erasers of DNA Methylation

A number of enzymes and proteins are involved in forming 5mC (writers), binding 5mC (readers), and removing 5mC (erasers). This section of the review will discuss the players involved in these processes and will review potential mechanisms of writing, reading, and erasing.

A. Writers

The writers of 5mC are DNA methyltransferases (DNMTs), which are enzymes that catalyze cytosine methylation in CGs. DNMT forms a covalent bond with the carbon at position 6 of the cytosine. DNMT catalyzes the transfer of a methyl group from *S*-Adenosyl-L-methionine (SAM), the universal methyl donor, to the carbon at position 5 of the cytosine. The transfer reaction releases DNMT from cytosine binding and the methyl group is now covalently bonded to the 5th carbon of cytosine forming 5mC (Jones and Baylin, 2007).

There are three DNMTs in mammals: DNMT1, DNMT3a, and DNMT3b. Another related protein, DNMT3L, has been identified but it lacks catalytic activity; however, it does participate in methylation by aiding DNMT3a/b. The most abundant DNMT in somatic cells is DNMT1. Newly synthesized double-stranded DNA (dsDNA) is hemimethylated, the parental strand contains the methyl modifications while the new daughter strand does not (Ooi et al., 2009, Miranda and Jones, 2007). DNMT1 is the maintenance methylase that is recruited to newly synthesized dsDNA helices to methylate the daughter strand (Sharif and Koseki, 2011, Jurkowska et al., 2011). DNMT1 can only methylate one strand at a time so it does not swap out its target strand while moving along its substrate. This allows the enzyme to follow DNA replication and methylate the daughter strand before the chromatin is reassembled (Hermann et al., 2004).

DNMT3a and DNMT3b (together will be referred to as DNMT3a/b) are *de novo* methyltransferases that are required for embryonic development in mammals. The DNA methylome is erased in early embryogenesis. DNMT3a/b establishes new methylation marks at the blastocyst stage and creates a new global DNA methylation landscape (Yang et al., 2012). The new methylome is inherited in somatic tissues by DNMT1 (Ooi et al., 2009). It has been reported that the DNMTs do not function independently and that DNMT3a/b works in conjunction with DNMT1 to maintain the methylation state of the genome. A new model for maintenance of the DNA methylome proposed by Jones and Liang states that localization of DNMT1 to the replication fork and interaction of DNMT3a/b with specific histone modifications are required for DNA methylation maintenance. This model was developed based on research which demonstrated that 30% of CGs remained hemimethylated in repeat sequences of the mouse genome when DNMT3a/b were knocked out. After DNMT1 methylates the newly synthesized DNA, it follows the replication fork down the DNA. DNMT3a/b is then recruited by specific proteins to methylate sites that were missed by DNMT1 (Jones and Liang, 2009). Thus, DNMTs work cooperatively to maintain the genomic methylation pattern.

B. Readers

The readers of DNA methylation are methylated DNA-binding proteins (MBPs) that bind to 5mC. The methyl-CpG-binding protein (MeCP2) is an MDP that can bind to a single methylated CG. It contains a methyl CpG-binding domain (MBD) and a transcriptional repression domain (TRD). While the MBD interacts with 5mC, the TRD recruits corepressor complexes containing histone deacetylases (HDACs) and Sin3a, a transcriptional repressor (Choudhuri et al., 2010). HDACs facilitate chromatin condensation by deacetylating lysines on surrounding histones resulting in a positively charged lysine with an increased affinity for the negatively charged DNA (Nan et al., 1998). Thus, the crosstalk between DNA methylation and histone modifications mediated by a reader, MeCP2, facilitates chromatin condensation and subsequent gene silencing. MeCP2 may also prevent activation directly by blocking the interaction of activators with the promoter (Choudhuri et al., 2010). It has also been reported that MeCP2 associates with histone methyltransferases to form H3K9 dimethylation (Histone 3 Lysine 9), which is a repressive histone modification (Fuks et al., 2003). This further promotes chromatin condensation of the region and gene silencing.

Along with MeCP2, other proteins that bind to 5mC include MBD1-4 and UHRF1. These proteins have been shown to play a role in methylation-dependent transcriptional repression (Wade, 2001) but some have been reported to function in other pathways, such as DNA repair. MBD4 aids in the repair of T/G mismatches due to the deamination of 5mC (Hendrich et al., 1999). Ubiquitin-like, containing PHD and RING finger Domains 1 (UHRF1) is a reader that works to recruit DNMT1. It preferentially binds to hemimethylated DNA via its Set and RING finger-associated (SRA) domain, which also interacts with HDAC1 to further promote silencing (Bostick et al., 2007).

C. Erasers

Reversibility of DNA methylation modifications is a controversial topic. An active DNA demethylating enzyme has not been discovered in mammalian cells; however, passive demethylation has been reported to occur via endogenous and exogenous sources. Erasure of DNA methylation occurs in early embryogenesis (Yang et al., 2012), aging (Murgatroyd et al., 2010), disease states such as cancer (Torano et al., 2012), and by chemical agents such as 5-aza-cytidin (Mateos et al., 2005). Remodeling of the DNA methylation profile in early embryogenesis is important for the development of the fetus and begins by removal of the parental methylome. Active demethylation of the paternal genome and passive demethylation of the maternal genome occurs soon after fertilization (Aguilera et al., 2010). Research involving mammalian pronuclear zygotes has shown that only a few hours after fertilization the paternal genome undergoes active demethylation in a replication-independent manner (Mayer et al., 2000, Park et al., 2007). To understand the complete mechanism of this process, further studies are required; however, it is believed that the active demethylation occurs indirectly. The process may occur through a DNA repair pathway such as base excision repair (BER) or nucleotide excision repair (NER) (Razin et al., 1986, Weiss et al., 1996). One theory suggests that 5mC is deaminated by deaminases to thiamine and the resulting T:G mismatch is repaired by thymine DNA glycosylase (TDG) and MBD4 (Hendrich et al., 1999).

In contrast to the active demethylation of the paternal genome, the maternal genome escapes this process and becomes demethylated through a passive mechanism. Demethylation of the maternal genome does not occur until the beginning of mitotic division. When mitosis begins to occur, both maternal and paternal genomes undergo passive demethylation that is replication-dependent (Rougier et al., 1998). The passive demethylation is thought to be due to the absence of DNMT1. The paternal genome may contain preferential binding sites for a

putative demethylase which would explain why it initially undergoes active demethylation while the maternal genome does not (Wu and Morris, 2001).

The role of Ten-eleven translocation (Tet) proteins in active demethylation may involve deaminases and enzymes involved in repair pathways. Tet proteins are members of a family of 2-oxoglutarate and Fe(II)-dependent dioxygenases that can catalyze 5mC oxidation which generates 5mC derivatives such as 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Thymine DNA glycosylase (TDG) recognizes 5fC and 5caC and removal of either of these derivatives results in an abasic site, which after repair generates unmethylated cytosine. Evidence to support this theory has been extensively reviewed in Wu and Zhang et al. Another proposed mechanism of Tet proteins' role in active demethylation involves deamination followed by DNA repair. Deamination of 5hmC by deaminases to 5-hydroxymethyluracil (5hmU) will be recognized by glycosylases in BER to correct the U:G mismatch and will replace 5hmU with unmethylated cytosine (Wu and Zhang, 2011). Conversion of 5mC to unmethylated cytosine mediated by Tet proteins, deaminases, and repair pathways may be one mechanism of active demethylation of the paternal genome after fertilization.

IV. THE LANDSCAPE OF DNA METHYLATION IN CANCER

Cancer cells have a unique methylome that differs from normal cells. Figure 1 summarizes key features of a cancer DNA methylome compared to the DNA methylome of a normal cell. Interestingly, both hyper- and hypomethylation can contribute to carcinogenesis. In general, cancer cells display gene-specific promoter hypermethylation and global genome hypomethylation. Whereas DNA hypermethylation can lead to the inactivation of tumor suppressor genes, DNA hypomethylation can activate proto-oncogenes and both of these events can lead to proliferation (Torano et al., 2012, De Smet and Lorient, 2013). This section will discuss the DNA methylation profile of cancer cells which differs from normal cells by both hyper- and hypomethylation events.

A. Hypermethylation in Cancer

The CGIs located in promoters are unmethylated in normal cells. Critical genes that are required for normal cell function such as tumor suppressor genes and tissue-specific genes are often inactivated in cancer cells due to hypermethylation of their promoters (Ooi et al., 2009, Miranda and Jones, 2007). Inactivation of these genes results in dysfunction of a variety of pathways such as DNA repair and cell cycle control contributing to carcinogenesis. Promoter hypermethylation can serve as the first or second hit in Knudson's two-hit model of carcinogenesis. If hypermethylation is the first hit, heterozygosity is established and dysfunction may result from haploinsufficiency. Aberrant promoter methylation of a gene whose other allele has been inactivated can result in loss of heterozygosity and expression will be fully silenced (Baylin, 2012).

Promoter hypermethylation of tumor suppressor genes such as *PI6*, *RB* (retinoblastoma), *MLH1* (mutL homolog1), *BRCA1* (breast cancer 1) and others have been reported in various types of human cancers (Esteller, 2008). Promoter hypermethylation of a specific tumor suppressor gene is a hallmark of some cancer types, such as hereditary non-polyposis colorectal cancer (HNPCC) and promoter methylation of *MLH1*, a gene that encodes a core mismatch repair protein (Herman et al., 1998). Other housekeeping genes that are constitutively expressed may also become silenced due to promoter hypermethylation. These genes which are involved in a wide spectrum of processes such as cell cycle control (Zang et al., 2011) and stress response (Hanada et al., 2012) are essential for cellular metabolism and inactivation of these genes aids carcinogenesis.

Tissue-specific genes are involved in cellular processes that are unique to a certain cell type. These genes can become deregulated in cancer due to aberrant promoter hypermethylation. For example, MASPIN is a gene which encodes the mammary serine protease inhibitor protein and it is only expressed in epithelial cells. Promoter hypermethylation silences this gene in a number of cancers such as colorectal cancer and thyroid cancer (Bettstetter et al., 2005, Boltze et al., 2003).

Tumor suppressor microRNAs (miRNAs), another mechanism of epigenetic regulation, may become inactivated due to aberrant promoter methylation in cancer cells. miRNAs are non-coding RNAs (ncRNAs) that regulate gene expression by binding to their target mRNA and inhibiting translation or inducing their degradation. Tumor suppressor miRNAs such as miR-15a and miR-16-1, which repress the anti-apoptotic protein Bcl2, can become deregulated in cancer due to promoter hypermethylation (Cimmino et al., 2005).

B. Hypomethylation in Cancer

Generally, cancer cells demonstrate a global decrease in 5mC (Torano et al., 2012). DNA hypomethylation can promote carcinogenesis via a number of mechanisms: activation of TEs (Yagi et al., 2012), activation of proto-oncogenes (Gokul and Khosla, 2012), loss of imprinting (Anwar et al., 2012), activation of viral DNA (Park et al., 2011), and activation of tissue-specific genes specific to other cell types (Cannuyer et al., 2013).

DNA hypomethylation is most tightly tied to carcinogenesis through its deregulation of TEs and pericentromeric regions that lead to genomic instability. In normal cells, CGs located in TEs and repetitive sequences such as long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), and Alu sequences are methylated and genomic integrity is preserved. Loss of methylation of TEs leads to their mobilization and integration into random parts of the genome which may result in genomic instability (Yagi et al., 2012). Hypomethylation of repetitive sequences such as LINEs, SINEs, and Alu sequences may result in an increase of mitotic recombination and compromise the genome's integrity. Pericentric regions of DNA may also become hypomethylated in cancer which will promote chromosomal rearrangements, mitotic recombination, and aneuploidy (Eden et al., 2003, Karpf and Matsui, 2005).

Another hypomethylation event associated with cancer is the loss of methylation on viral sequences. Viral DNA integrated into the host's genome is methylated and repressed. Loss of methylation of these latent viral sequences can lead to tumor promotion and progression (Park et al., 2011). For example, cervical cancer progression can be caused by loss of methylation of the genital human papillomavirus (HPV) genome which is normally repressed by DNA methylation (Kulis and Esteller, 2010).

Activation of tissue-specific genes specific to other cell types occurs in cancer (Cannuyer et al., 2013). Germ cell-specific tumor antigen genes such as the MAGE, BAGE, LAGE, GAGE gene families are repressed in somatic tissues by DNA methylation and loss of methylation activates these genes in some types of malignant human cancers. Expression of these genes in cancer leads to immune rejection by inducing production of tumor-specific antigens (Weber et al., 2007).

The mechanism of hypomethylation seen in cancer is poorly understood. Some theories suggest that catalytically inactive variants of DNMT3b mediate the hypomethylation events. Many variants of this enzyme lacking a catalytic domain have been identified in numerous cancer cell lines. These variants are suspected to negatively regulate DNMT activity and compete with the active form to bind to target DNA sequences (Kulis and Esteller, 2010).

V. DNA METHYLATION AND METAL-INDUCED CARCINOGENESIS

Carcinogenic metals are ubiquitous elements and humans are exposed to these toxicants via air, drinking water, occupational settings, and consumer products. A great number of epidemiological studies have established the carcinogenicity of metals by associating metal exposure with human cancer incidence. In this next section, we will discuss some of the human, animal and *in vitro* evidence that links nickel (Ni), arsenic (As), cadmium (Cd), and hexavalent chromium [Cr (VI)] with cancer in order to emphasize the critical concern of human exposure to these agents. Many of the investigations linking these metals with cancer have only observed a significant association with the highest dose used. It is important to remember when reviewing the literature that detecting statistically significant results in small, by necessity, animal populations is a challenge; however, because animal bioassay populations are usually genetically identical, significance is achieved at lower population sizes compared to human populations. Supplementary table 2 provides empirical data and pays close attention to the doses used in animal bioassay studies that describe the carcinogenic outcomes of Ni, As, Cd and Cr (VI) exposures.

Key features of the distinctive DNA methylome observed in cancer cells offers potential for the establishment of new biomarkers for the identification of different cancer types. Many exogenous chemicals can perturb the methylome and promote carcinogenesis. Certain compounds containing nickel, arsenic, cadmium, and Cr (VI) are known human carcinogens that alter DNA methylation patterns to resemble the methylome of a cancer cell. Exposure to compounds containing these metals occurs throughout the world by a variety of sources.

Altering the DNA methylation status is one of the underlying mechanisms that characterize the carcinogenicity of metal compounds. While many of the carcinogenic metal compounds have shown to be weak mutagens, they influence gene expression via epigenetic mechanisms (Arita et al., 2009). Paradoxically, metals have been shown to turn off tumor suppressor genes by inducing promoter hypermethylation, while at the same time, causing genome-wide hypomethylation. It is important to note here that methyl modifications are not the only epigenetic mark that is altered by metals; metal exposure may also lead to changes in histone modifications that further promote carcinogenesis (Arita and Costa, 2009).

Nickel

A. Nickel Exposure—Nickel is a metal that is the twenty-fourth most abundant element found in the earth's crust. Nickel can also be found in volcanic eruptions, soils, ocean floors, and ocean water (Kargacin et al., 1993, Cruz et al., 2006). Compounds containing nickel have been found to cause oxidative stress (Schmid et al., 2007), hematotoxicity (Salnikow et al., 2003), and immunotoxicity (Hostynek, 2006). Exposure to nickel is an environmental concern because nickel can be found in many products such as coins, jewelry, stainless steel, batteries, medical devices, and carbon particles. Occupational exposure to nickel compounds occurs during nickel refinery, plating, and welding operations (Salnikow and Zhitkovich, 2008). It has long been understood that nickel compounds are an important human toxicant that has the ability to induce tumorigenesis. Nickel refinery workers have shown a high incidence of lung, nasal, and pharyngeal cancers (Sunderman, 1984). *In vitro* mutation assays in *Salmonella* and mammalian cells have shown that nickel compounds have low mutagenic capabilities (Kargacin et al., 1993); therefore, its role in carcinogenesis is thought to be largely through an epigenetic mechanism.

B. Human, animal and *in vitro* investigations—Nickel compounds, inclusive of water-soluble salts, are known human carcinogens and occupational exposure to these compounds is a concern (Arita et al., 2009). Various human, animal and *in vitro* investigations have provided data indicating the toxic effects of nickel compounds

establishing the hazard of human exposure to nickel. Table 1 summarizes the human, animal and *in vitro* investigations discussed in this section.

The largest concern for human exposure to nickel is from occupational exposure. A study by Qayyum et al. investigated cellular toxicity of buccal cells taken from subjects occupationally exposed to nickel and Cr (VI) in the electroplating industry. The study found that the frequency of micronuclei was higher in the exposed population when compared to the unexposed control population and micronuclei frequency increased as duration of exposure increased in the exposed groups. Also, plasma nickel and chromium levels showed a positive correlation with frequency of micronuclei (Qayyum et al., 2012). An investigation by Sorahan and Williams examined 812 Welsh nickel refinery workers who had worked at the refinery for at least 8 years. The study found that the overall lung cancer mortality rate was increased compared to the general population. For individuals who had been working at the refinery for more than 20 years, lung cancer risk was significantly elevated (Sorahan and Williams, 2005).

Occupational exposure to nickel has also been associated with alterations in global levels of histone modifications in peripheral blood mononuclear cells (PBMCs). 45 Chinese nickel refinery workers and 75 referents were examined in a study by Arita et al. The group found that H3K4 trimethylation was elevated and H3K9 dimethylation was decreased in PBMCs of nickel-exposed subjects compared with referents (Arita et al., 2012).

Oral cancer incidence occurs at high frequencies in Taiwan. Changhua County, located in central Taiwan, has one of the highest rates of oral cancer in the country and this region also contains many metal-related industries and metal-contaminated soil. A study found that blood levels of nickel were significantly higher in oral cancer patients compared to control patients indicating that exposures to nickel in this region of Taiwan may play a role in the development of oral cancer (Yuan et al., 2011).

Nickel has also demonstrated its toxicological and carcinogenic potential in various animal bioassay studies. One of the earliest studies examining nickel carcinogenicity in animals found that rats exposed by inhalation to nickel sulfide (NiS) for 78 weeks formed significantly more lung tumors than rats exposed to filtered air. Rats were exposed in a 3.85 m³ chamber with a dose averaging 0.97 mg/m³ throughout the 78 weeks. 14% of exposed rats and 0.1% of control rats formed tumors (Ottolenghi et al., 1975). Dunnick et al. exposed male and female F344 rats via inhalation to nickel subsulfide (Ni₃S₂) (0.15 and 1 mg/m³) and nickel oxide (NiO) (0.62, 1.25 and 2.5 mg/m³) for 2 years. Results of the study revealed that both nickel compounds caused an increased amount of lung neoplasms in male and female rats. 4, 24 and 40% of rats exposed to 0, 0.15 or 1.0 mg/m³ Ni₃S₂, respectively, formed neoplasms in the lungs. 4, 2, 23 and 17% of rats exposed to 0, 0.62, 1.25 or 2.5 mg/m³ NiO, respectively, formed lung neoplasms. Only the higher doses of nickel compounds caused significantly more tumors in exposed rats than unexposed rats (Dunnick et al., 1995).

Wistar rats exposed by whole body inhalation to various concentrations of metallic nickel (nickel metal powder) (0.1 and 0.4 mg/m³) for 2 years displayed lung alterations associated with the exposure. Various lung alterations were seen following euthanasia: proteinosis, alveolar histiocytosis, chronic inflammation, and bronchiolar/alveolar hyperplasia. Male rats exposed to 0.4 mg Ni/m³ displayed a dose-dependent increase in adrenal gland pheochromocytomas (Oller et al., 2008).

Nickel chloride is a cocarcinogen with ultraviolet radiation (UVR). Female hairless mice, Skh1 line, were exposed to UVR (1.0 kJ/m²– 3/wk) + various concentrations of nickel chloride (NiCl₂) in drinking-water (20, 100 and 500 mg/l) for 26 weeks. Only the mice

exposed to the highest doses (100 and 500 mg/l NiCl₂) produced significantly more skin tumors than control mice (Uddin et al., 2007). This may have implications for humans who are occupationally exposed to nickel and spending increased amounts of time in the sun.

Previous *in vitro* investigations involving nickel have demonstrated its ability to induce DNA damage (Pool-Zobel et al., 1994), cell transformation (Conway and Costa, 1989), oxidative stress (Huang et al., 1993), and epigenetic alterations (Arita et al., 2009). Earlier studies by Biedermann et al. and Miura et al. suggested that mutagenesis does not characterize nickel's carcinogenic potential. Biedermann et al. found that nickel subsulfide induces cytotoxicity and anchorage independent growth in cultured human diploid fibroblasts without mutagenesis (Biedermann KA, 1987). Miura et al. demonstrated that nickel subsulfide and green nickel oxide induced morphological transformation of mouse embryo cells without inducing mutations that confer ouabain resistance (Miura T, 1989). Some more recent *in vitro* findings have demonstrated nickel's ability to induce apoptosis of human neutrophils and inhibit the histone demethylase JMJD1A which leads to repression of Spry2.

A study by Freitas et al. found that nickel induces apoptosis in human neutrophils. Treatment of human neutrophils with 250 and 500 μ M nickel nitrate caused an increased amount of apoptosis. When cells were also treated with an NADPH oxidase inhibitor, Ni (II)-induced apoptotic levels returned to control levels. These findings indicate that Ni (II) can induce apoptosis of neutrophils and this event may be dependent on NADPH oxidase-derived reactive oxygen species (Freitas et al., 2012).

Nickel inhibits the histone demethylase JMJD1A and represses Spry2 in human bronchial epithelial cells. Spry2 is a key regulator of receptor tyrosine kinase/extracellular signal-regulated kinase (ERK) signaling and a JMJD1A-targeted gene. By inhibiting JMJD1A, nickel increased the level of H3K9 dimethylation, a repressive histone modification, at the Spry2 promoter which led to Spry2 repression (Chen et al., 2010b, Chen et al., 2010a).

The investigations discussed above reveal nickel's potential toxic and carcinogenic effects on both cellular and organism levels. To further review the carcinogenicity of nickel and provide possible mechanisms of nickel-induced carcinogenesis, we will discuss the ability of this metal to induce changes in the methylome that are also seen in carcinogenesis.

C. DNA Methylation and Nickel-Induced Carcinogenesis

i. Hypermethylation: One of the first experiments to demonstrate nickel's influence on DNA methylation was done in the Chinese hamster cell line (G12). The xanthine guanine phosphoribosyl transferase gene (*gpt*) was silenced by treatment with 2 μ g/cm² NiS for 16h, but it was reactivated after treatment with the demethylating agent 5-azaC. A methylation assay using the restriction enzyme HaeII revealed that the gene silencing was in part due to an increase in DNA methylation. The increased DNA methylation along with the location of the gene relative to heterochromatin was associated with the silencing of the *gpt* gene (Lee et al., 1995). This research provided evidence of nickel's ability to silence genes via an increase in DNA methylation and alluded to the notion that nickel-induced carcinogenesis may be partly caused by promoter hypermethylation of tumor suppressor genes.

Another study using the transgenic mammalian G12 cell line demonstrated that nickel can play a role in the spreading of heterochromatin. Nickel was able to induce the *gpt* gene incorporation into heterochromatin only when the *gpt* gene was placed near a heterochromatic region as seen in the G12 line. *Gpt* in the G10 line was not silenced via incorporation into heterochromatin because this cell line has the *gpt* gene in euchromatin away from a heterochromatic region (Lee et al., 1995, Klein and Costa, 1997).

Later, it was shown that nickel-induced heterochromatinization was caused by nickel displacing magnesium in heterochromatic complexes. Magnesium complexes with DNA in the phosphate backbone to promote condensation. Ni^{2+} displaces Mg^{2+} and increases the level of chromatin condensation much more effectively than magnesium (Ellen et al., 2009). DNMTs could be signaled somehow by condensation events. Therefore, nickel causes gene silencing first by heterochromatin spreading and subsequent methylation of those genes taken into heterochromatin. If the genes silenced are tumor suppressor or senescence genes, then carcinogenesis could be a result.

Nickel has been associated with the hypermethylation of a number of genes *in vivo*. *P16* gene encodes a tumor suppressor involved in cell cycle regulation and is often silenced in many human cancers. Wild type C57BL/6 mice and mice heterozygous for the tumor suppressor gene *P53* were implanted with NiS. Both wild type and *P53* heterozygous mice developed tumors with the *P16* gene hypermethylated, as well as activation of the mitogen activated protein kinase (MAPK) signaling pathway. (Govindarajan et al., 2002). *RAR β* and *RASSF1A* are genes that encode tumor suppressors that mediate cell growth and induce cell cycle arrest, respectively. Wistar rats given an intramuscular injection of 10 mg nickel subsulfide developed muscle tumors that showed 5' hypermethylation of *RAR β* , *RASSF1A*, and *P16* (Zhang et al., 2011). The O⁶-methylguanine DNA methyltransferase (*MGMT*) gene, which encodes an enzyme that repairs O⁶-methylguanine, was hypermethylated in NiS-transformed human bronchial epithelial (16HBE) cells. Cells were treated with 1 or 2 $\mu\text{g}/\text{cm}^2$ NiS for 24h on two or three separate occasions. Both treatments induced hypermethylation of the *MGMT* promoter 6 days after treatment. Repression of *MGMT* by promoter hypermethylation was confirmed by reduced to undetectable levels of both mRNA and protein. The investigation also identified concurrent binding of MeCP2 and DNMT1 to the CpG island of the *MGMT* promoter (Ji et al., 2008).

ii. Hypomethylation: Genome-wide DNA hypomethylation has been reported in nickel-induced carcinogenesis. A line of human bronchial epithelial cells, 16HBE, was treated with 1 and 2 $\mu\text{g}/\text{cm}^2$ NiS for 24h. A 5mC immunofluorescence assay showed that the fluorescence intensity of NiS-induced transformed cells decreased. An Sss1 methylase assay confirmed that NiS-treated cells contained a lower amount of 5mC than control cells (Yang et al., 2010).

D. Discussion—Based on the evidence in Table 1, nickel exposure should be a major concern for human health. Its ability to form tumors in animals and its large epidemiological association with lung cancer establishes this metal as a human carcinogen. Human exposure concerns arise from the natural occurrence of nickel compounds in the environment and the use of nickel in many occupational settings. Considering the low mutagenicity of nickel compounds, significant attention must be given to epigenetic mechanisms in order to fully characterize nickel's carcinogenic potential. The research performed on the epigenetic effects of nickel provides evidence of nickel's ability to induce DNA methylation alterations that characterize the cancer methylome- promoter hypermethylation of cancer-related genes and global hypomethylation.

Epigenetic effects can also influence mutagenesis. Ji et al. reported nickel-induced hypermethylation of the *MGMT* promoter, a DNA repair protein that repairs alkylation of the 6' oxygen on guanine residues. Coinciding with this report, Iwizki et al. found that Ni^{2+} inhibits the repair of O⁶-methylguanine in mammalian cells in a dose dependent manner (Iwizki F, 1998). Once a key DNA repair protein is silenced, the cell may acquire a "mutator phenotype". Simply put, loss of expression of *MGMT* may greatly increase the mutation rates at other loci. *MGMT* suppression and hypermethylation may be an early event in nickel-induced carcinogenesis since the effects observed in Ji et al. occurred only 6 days

after NiS treatment. Thus, we can hypothesize that an early event driving nickel-induced carcinogenesis may be the induction of a mutator phenotype via hypermethylation of *MGMT* promoter.

Nickel-induced repression of tumor suppressor genes due to promoter hypermethylation have been reported in a number of studies but a mechanism for nickel-induced hypermethylation is still vague. Early investigations of the epigenetic effects of nickel report nickel displacing magnesium in the DNA backbone leading to DNA condensation and subsequent hypermethylation (Ellen et al., 2009). This theory has not been tested for nickel-induced silencing of tumor suppressor genes away from heterochromatin. Lee et al. found that nickel did not induce condensation in the *gpt* gene when placed away from heterochromatin. However, this was an *in vitro* study using a transgenic cell line, whereas many nickel-induced hypermethylated tumor suppressor genes have now been found *in vivo*. Ji et al. located MeCP2 and DNMT1 at the promoter of *MGMT* in NiS-treated cells. Coinciding with these findings, Yan et al. found that the *gpt* gene contained hypoacetylated histones and MeCP2 was targeted to the *gpt* gene loci (Yan Y, 2003). MeCP2 recruits HDACs and corepressor complexes, such as sin3a. Nickel's displacement of magnesium in the DNA backbone may initiate chromatin condensation and recruit DNMT1 to hypermethylate the site. Hypermethylation recruits MeCP2 to bind 5mC which will signal HDACs and corepressors that further facilitate complete condensation. Thus, a model describing a possible mechanism of nickel-induced carcinogenesis can be proposed (fig. 2).

Further research examining the methylome of humans exposed to nickel compounds is needed to determine hallmark nickel-induced DNA methylation alterations and exploit these alterations in cancer therapy. While nickel's ability to induce aberrant methylation marks has been well documented in the literature, very little data is available regarding nickel's tendency to decrease genomic methylation levels. Research aiming to further investigate nickel's ability to lower global methylation levels is required in order to fully characterize this metal's epigenetic effects. A mechanism describing nickel-induced global hypomethylation that coincides with its mechanism of hypermethylation has yet to be unearthed and should be explored in future research.

Arsenic

A. Arsenic Exposure—Arsenic is a metalloid listed on the Agency for Toxic Substances and Disease Registry (ATSDR) Priority List of Hazardous Substances and it is a human carcinogen. Arsenic exposure has been associated with skin, lung, liver and bladder cancers (Jensen et al., 2008) as well as noncarcinogenic outcomes, including cardiovascular disease and neurological deficits (Tseng et al., 2005, Wasserman et al., 2004). Arsenic displays both acute and chronic toxicity and has no known biological function. The most common mode of human exposure to inorganic arsenic is through contaminated drinking-water. It affects more than 140 million people in more than 70 countries (Kinniburgh and Kosmus, 2002).

The two most abundant forms of inorganic arsenic are arsenate, As (V), and arsenite, As (III). Once inorganic arsenic gets absorbed, it can be biotransformed to organic arsenic (Aposhian, 1997). As (III) is more toxic than As (V), but As (V) can be reduced to As (III) in the body. Although methylation of arsenic promotes excretion, the methylated (organic) form is more toxic. Monomethylarsonous acid, MMA(III), is far more toxic than its parent compound and it is of particular interest in arsenic-mediated toxicity (Styblo et al., 2002). Arsenic is a poor mutagen so its carcinogenicity is thought to occur through epigenetic mechanisms.

B. Human, animal and *in vitro* investigations—Here we will discuss some of the human, animal and *in vitro* investigations that characterize arsenic's carcinogenicity. These investigations are summarized in table 2.

Various epidemiological studies have linked arsenic with cancer incidence in humans. A study by Meliker et al. associated arsenic concentrations exceeding 300–500 µg/l in drinking-water with bladder cancer incidence but they did not find an association with arsenic and cancer at concentrations below 200 µg/l (Meliker JR, 2007).

An ecological study investigated arsenic exposure in an area of Taiwan, China where Blackfoot disease was an endemic. Blackfoot disease is a unique peripheral vascular disease caused by chronic ingestion of arsenic from drinking-water. The study found that people living in the area of Taiwan where Blackfoot disease was prevalent had a significantly higher mortality from bladder and kidney cancers compared to the general population of Taiwan. The arsenic concentration in the well-water in this region of Taiwan ranged from 350 to 1140 µg/l. The standardized mortality ratio for bladder cancer and kidney cancer increased with the prevalence of Blackfoot disease (Chen et al., 1985).

A study by Kurttio et al. investigated well-water arsenic in Finland and kidney and bladder cancer incidence. Arsenic concentrations in the well-water were low, 0.1 µg/l – 64 µg/l. The group did not find an association between arsenic and kidney cancer but did find an association between low, chronic arsenic exposure and bladder cancer risk (Kurttio et al., 1999).

Epidemiological studies have also associated arsenic with skin cancer incidence. A case-control study performed by Leonardi et al. aimed to investigate the risk of skin cancer with a low arsenic exposure. The study examined districts of Hungary, Romania, and Slovakia and found a positive association between basal cell carcinoma and drinking-water arsenic with concentrations >100 µg/l (Leonardi et al., 2012). Drinking-water in Bangladesh is highly contaminated with arsenic. An estimated 40 million people are at risk of arsenic poisoning-related diseases because of the arsenic-contaminated groundwater. Over half of the wells in the southern deltaic regions contain arsenic concentrations over 100 µg/l. A survey done by the SOES/DCH found that 21% of people drinking from the arsenic-contaminated wells had arsenical skin lesions (Winston H. Yu, 2003).

Wisconsin's Fox River Valley contains drinking-water wells with high arsenic concentrations. 11% of the wells contain arsenic above 20 µg/l, which is above the US drinking-water standard of 10 µg/l. A study conducted by the Wisconsin Division of Public Health found that incidence of skin cancer was increased for residents over the age of 35 who had consumed arsenic-contaminated water for at least 10 years (Knobeloch et al., 2006).

Arsenic has also displayed its toxic and carcinogenic potential in a number of animal models. Male A/J mice exposed to 1, 10 and 100 mg/l sodium arsenate (Na_2HAsO_4) via drinking-water for 18 months demonstrated an increase in lung tumor multiplicity and lung tumor size. The percentage of tumors with size more than 4 mm was increased in a dose-related manner in the exposed mice compared to the control mice (Cui et al., 2006a). Wei et al. treated male F344 rats with 12.5, 50 or 200 mg/l dimethylarsinic (DMA) in drinking-water for up to 2 years. Only rats exposed to the higher doses (50 and 200 mg/l) formed urinary bladder lesions. There was a 39% and 45% incidence of urinary bladder lesions in mice exposed to 50 and 200 mg/l DMA, respectively (Wei et al., 2002). The liver is another target for arsenic-induced carcinogenesis. Male F344 rats were treated with 50 and 200 mg/l trimethylarsine oxide (TMAO) in the drinking-water for 2 years. Incidences of

hepatocellular adenomas were 14, 24 and 36% in mice exposed to 0, 50 and 200 mg/l TMAO, respectively. Only rats treated with 200 mg/l TMAO formed significantly more liver tumors than control rats (Shen et al., 2003).

An early *in vitro* study by Biedermann et al. demonstrated the carcinogenic potential of arsenic compounds. The group found that sodium arsenite and sodium arsenate induced cytotoxicity and anchorage independent growth in human diploid cells (Biedermann KA, 1987). *In vitro* studies have suggested that arsenic may mediate its carcinogenic effects by promoting an apoptosis-resistant phenotype. A study by Pi et al. found that HaCaT cells (immortalized human keratinocytes) grown in low concentrations of sodium arsenite (100 nM) for 28 weeks displayed resistance to apoptosis. When the arsenic-treated cells were exposed to ultraviolet A irradiation (UVA), the cells resisted an apoptotic response (Pi et al., 2005). Arsenic is a cocarcinogen with UV in mice. Arsenic suppression of apoptosis allows UV-induced DNA damaged cells to survive which further fuels carcinogenesis by allowing the growth of cells containing damaged DNA. It was reported by Wu et al. that short-term exposure to arsenite can also reduce the amount of apoptosis. Mouse keratinocytes, 291.03C line, were exposed to sodium arsenite for 24h and immediately irradiated with 0.30 kJ/m². UVR-induced apoptosis at 24 hr was decreased by 23% and 62% at 2.5 μ M and 5.0 μ M arsenite, respectively (Wu et al., 2005). Lack of p53 function has been hypothesized to play a role in arsenic-induced inhibition of apoptosis (Chen et al., 2005).

C. DNA Methylation and Arsenic-Induced Carcinogenesis

i. Hypermethylation: Several studies have associated arsenic with gene-specific hypermethylation. The methylation status of the *P53* and *P16* genes was analyzed in DNA from blood samples from subjects chronically exposed to arsenic in West Bengal, India. *P53* gene encodes a tumor suppressor that promotes apoptosis and represses the cell cycle. Hypermethylation of the *P53* and *P16* promoter regions was found in more arsenic-exposed subjects compared to that of unexposed controls (Guha Mazumder and Dasgupta, 2011). Another study compared *P53* promoter methylation between patients who had arsenic-induced skin cancer and patients who had skin cancer unrelated to arsenic. There was a significantly higher degree of hypermethylation of *P53* gene in arsenic-induced skin cancer tissues compared to tissues of patients unexposed to arsenic (Chanda et al., 2006). One study reported that *P16* promoter hypermethylation appeared more frequently in DNA extracted from white blood cells of patients with arseniasis than healthy controls (Zhang et al., 2007). In addition to *P53* and *P16*, the death-associated protein kinase (*DAPK*) gene, which encodes a tumor suppressor that is a positive mediator of gamma-interferon induced apoptosis, became silenced due to promoter hypermethylation in human uroepithelial cells after treatment with As (III) (Huang et al., 2009).

An increase in global 5mC is not a characteristic of cancer, but it has been reported to occur after arsenic exposure. Majumdar et al. examined peripheral blood mononuclear cells of 64 subjects exposed to arsenic via drinking-water. The study indicated an increase of genomic methylation in subjects exposed to 250–500 μ g/l As. The global hypermethylation was lost in subjects exposed to greater than 500 μ g/l As (Majumdar et al., 2010). Jensen et al. demonstrated that arsenic induces aberrant methylation in gene promoters on a global level. Human uroepithelial cells were treated with either As (III) or MMA(III) and 13,000 gene promoters were examined. The study found that arsenic caused hypoacetylation of histone 3 in promoters and a subset of these promoters displayed hypermethylation (Jensen et al., 2008).

ii. Hypomethylation: Global DNA hypomethylation is an early event in some cancers and occurs in response to arsenic exposure. Arsenic can cause liver cancer in mice and it is also

a potential target in humans; it has been associated with hepatocellular carcinomas and other hepatic lesions in humans (Mazumder, 2005, Centeno et al., 2002). DNA hypomethylation can increase gene expression especially when it occurs on the CGIs of the promoter. A study was done that exposed male mice to 45mg/l As (III) for 48 weeks via drinking-water and their gene expression and DNA methylation levels were analyzed. A methyl acceptance assay revealed that the mice exposed to As (III) had a decrease in hepatic DNA methylation levels. A microarray analysis of the liver samples showed aberrant gene expression with hepatic estrogen receptor- α (ER- α) having a markedly increased expression. A methylation-specific PCR (MSP) revealed that As (III) caused a reduction in the 5mC levels of the promoter region of ER- α . The chronic arsenic exposure also induced hepatic steatosis in the mice (Chen et al., 2004). Xie et al. examined the level of hepatic DNA methylation in newborn males who had mothers that were given a carcinogenic dose of arsenic. The metal was found at significant levels in the livers of the newborns after birth. The study found that global methylation levels of hepatic DNA remained constant, while a significant reduction in methylation occurred in CG-rich regions (Xie et al., 2007).

Nohara et al. demonstrated that arsenic's effect on global DNA methylation may be sex-dependent. The study found that male C57BL/6 mice treated with As (III) and/or a methyl-deficient diet had a decrease level of 5mC in the liver while females did not show this decrease. Surprisingly, females had a significantly increased amount of 5mC content in the liver when treated with As (III) + methyl-deficient diet (Nohara et al., 2011). Another group used a methyl-deficient diet along with As (III) exposure to reduce the frequency of methylation at several cytosine sites within the promoter region of the *Ha-ras* oncogene in hepatic DNA of C57BL/6J male mice. This same study also showed that As (III) could induce genomic hypomethylation in a dose dependent manner (Okoji et al., 2002).

Arsenic trioxide (As₂O₃) has been used effectively in the treatment of some cancers and its therapeutic effect is mediated by hypomethylation of tumor suppressor genes. A study conducted by Shen et al. found that As₂O₃ induced expression of anti-oncogene *hdpr1* mRNA in Jurkat cells, an acute lymphoblastic leukemia cell line. A methylation-specific PCR (MSP) revealed that the treated cells contained an *hdpr1* gene that was hypomethylated (Shen et al., 2010). Two studies demonstrated that As₂O₃ could activate the expression of *P15* gene, which encodes a protein that inhibits G1-S phase progression. MUTZ-1 cells (human myelodysplastic syndrome cells) treated with As₂O₃ reactivated *P15* by inducing hypomethylation. The researchers hypothesized that the hypomethylation was caused by arsenic-induced demethylation and/or inhibiting DNMT3a/b (Tong and Lin, 2002). Jin et al. found that As₂O₃ could activate expression of the *P15* gene by causing hypomethylation of the gene in Molt4 cells, an acute lymphoblastic leukemia cell line. The reactivated *P15* inhibited G1-S phase progression (Jin et al., 2001).

D. Discussion—Conflicting studies reporting both arsenic-induced hyper- and hypomethylation events on both global and gene-specific levels leave open the question regarding what arsenic actually does to DNA methylation levels. Mechanisms underlying these actions are also unexplained. Differences in methodology, model organisms, arsenic compounds, genomic locations, data interpretation, and randomness may explain the diverse results seen in studies examining arsenic's effect on the methylome.

Moreover, arsenic may be perturbing other epigenetic mechanisms such as histone modifications that further influence DNA methylation levels. Repressive histone modifications of tumor suppressor genes such as *P53* and *P16*, which have displayed promoter hypermethylation after arsenic treatment, may be present in nucleosomes surrounding the genes and influencing methylation status of the region. Future studies

investigating promoter hypermethylation induced by arsenic should also examine histone modifications surrounding the gene of interest.

Arsenic metabolism has a large potential to interfere with DNA methylation mechanisms because the SAM/methyltransferase pathway for biotransformation of arsenic overlaps with the DNA methylation pathway. As discussed in a previous section, DNMT catalyzes the transfer of a methyl group from SAM, the universal methyl donor, to the carbon at position 5 of the cytosine residue. The shared use of SAM in these pathways may explain the paradoxical effects induced by arsenic. Majumder et al. referenced above reported global hypermethylation in subjects exposed to 250–500 µg/l As but hypermethylation was no longer statistically significant in subjects exposed to greater than 500 µg/l As. Maintenance methylation may have been disrupted in the last group due to the large amount of arsenic that needed to be metabolized and excreted. The high arsenic concentration depleted cellular SAM levels which resulted in less cytosine methylation- hence the hypermethylation was lost in the >500 µg/l group.

Chen et al. referenced above is another case where extremely high arsenic levels result in a decrease in methylation. The group found that mice exposed chronically to 45 mg/l As developed hepatic steatosis which is a state linked to methyl-deficient diets. The high concentrations of arsenic in the mice may have depleted methyl stores due to arsenic metabolism which resulted in decreased methylation levels and induction of a methyl-deficient state.

The hypothesis that genomic hypomethylation is due to SAM depletion is weakened by the fact that SAM is normally at a high concentration (70 µM) within the cell and huge reserves always maintain SAM levels. A study conducted by Benbrahim-Tallaa et al. found that in an arsenic methyltransferase (AS3MT)- deficient cell line, RWPE-1, arsenic treatment still induced genomic hypomethylation. DNMT activity decreased while DNMT mRNA levels remained constant (Benbrahim-Tallaa et al., 2005). These facts support an alternative theory that arsenic-induced hypomethylation is SAM independent and occurs primarily via inhibition of DNMT activity.

Various reports have supported the theory that arsenic-induced hypomethylation is mediated by inhibiting DNMT activity. As (III) has been shown to repress DNMT1 and DNMT3A expression *in vitro* (Rossman and Klein, 2011). A study showed that DNMT activity was decreased by 40% in cells chronically exposed to a low-dose of As (III) for 18 weeks, however the DNMT1 mRNA expression was up-regulated in these same cells (Vahter, 2009). Another study contradicted this report by demonstrating that DNMT1 mRNA was decreased in cells exposed to As (III), but the DNMT enzymatic activity was still inhibited in these cells (Cui et al., 2006b). The loss of DNMT activity may be due to an enzymatic inhibition that is irreversible or a decrease in DNMT levels.

Whether or not gene-specific hypermethylation and global hypomethylation occur simultaneously during arsenic-induced transformation is not fully understood. One study that gave this possibility strength treated rat liver epithelial cells (TRL 1215) with arsenic for 18 weeks. The cells were injected into nude mice and formed tumors that correlated with DNA hypomethylation and elevated expression of the oncogene *c-Myc*. The transformed cells also displayed repression of *P21*, a tumor suppressor gene, due to hypermethylation (Liu et al., 2006). Further research investigating the mechanism of arsenic-induced hypomethylation and hypermethylation must be conducted in order to fully understand why and when both of these phenomena occur.

It is important to note here that other events play a role in arsenic-induced carcinogenesis. DNA metabolism (Rossman and Klein, 2011), generation of oxidative stress (Chervona and Costa, 2012), inhibition of DNA repair (Ebert et al., 2011), alteration of histone modifications (Chervona et al., 2012) and modulation of signal transduction pathways (Druwe and Vaillancourt, 2010) may also contribute to arsenic-induced cancers. Investigations aiming to examine the interactions of these events are necessary in order to fully understand arsenic's carcinogenic mechanisms.

Cadmium

E. Cadmium Exposure—Human exposure to cadmium occurs through the ingestion of cadmium-contaminated food and environmental tobacco smoke (Arita and Costa, 2009). Cadmium is a widely used heavy metal in industry, which affects human health through occupational exposure (Satarug et al., 2003). Cadmium can be released into the environment via natural activities, such as volcanic activity and river transport. Human activities, such as tobacco smoking, mining, smelting, and fossil fuel combustion can also contribute to environmental exposure to cadmium. Cadmium exerts its toxicity on the kidney, the skeletal system and the respiratory system. Although cadmium has shown to be genotoxic at high levels that arrest cell growth (Misra et al., 1998), its potential to cause mutations is limited. Cadmium does not participate in Fenton-like reactions which produce reactive oxygen species (ROS) that damage the DNA, although it may cause oxidative stress through inhibition of antioxidant enzymes (Abalea et al., 1999). Also, cadmium does not form DNA adducts (Waalkes and Poirier, 1984). Due to these observations, cadmium is considered an epigenetic carcinogen.

B. Human, animal, and *in vitro* investigations—Cadmium is a known human carcinogen. Table 3 summarizes some of the human, animal and *in vitro* investigations that characterize cadmium's carcinogenicity.

Epidemiological studies have linked cadmium exposure to cancer. Cadmium-contaminated food has become a major human health concern since cadmium is widely dispersed through the environment. A cohort study following over 41,000 men for 11 years investigated the effects of dietary cadmium exposure on prostate health. The study found that multivariable-adjusted dietary cadmium exposure was positively associated with overall prostate cancer. 7.5% of study participants developed prostate cancer with 29% of those cases localized and 26% advanced (Julin et al., 2012).

Cadmium may play a role in liver carcinogenesis. According to the US NHANES III, long-term cadmium exposure is associated with pre-diabetes and diabetes in a dose-dependent manner. Cadmium was associated with hepatocellular carcinoma (HCC) in an ecological study and cadmium-exposed mice developed HCC. It has been hypothesized that cadmium increases the risk of HCC by inducing hepatogenous diabetes (Satarug, 2012).

A study by Adams et. al. investigated an association between cadmium exposure and cancer mortality by examining prospective data in the NHANES III cohort. The study examined 9388 men and 10636 women. The group found that urinary cadmium levels were associated with overall cancer mortality in men and women. The association of urinary cadmium and lung cancer mortality in men was statistically significant. 23 men in the cohort died of pancreatic cancer and 17 of the men were in the upper quartile for urinary cadmium. There was also associations of urinary cadmium with lung, uterine, and ovarian cancer in women (Adams et al., 2012). Another study examined a possible association between cadmium and lung cancer by following two groups of participants living in either an area with high cadmium exposures or low cadmium exposures from 1985–2004. Urinary cadmium excretion and cadmium in garden soil were used as exposure indicators. The study found

that overall cancer was increased in the high exposure group and cadmium exposure was associated with lung cancer risk (Nawrot et al., 2006)a.

Cadmium has induced tumors in animals as seen in various animal bioassay studies. One of the earliest studies that examined cadmium carcinogenesis in animals was performed by Takenaka et al. The group exposed Wistar rats (TNO/W75) to 12.5, 25 or 50 $\mu\text{g}/\text{m}^3$ cadmium chloride (CdCl_2) via inhalation for 23 h/day, 7 days/wk for 18 months. The group found that 52 and 71% of rats exposed to 25 and 50 $\mu\text{g}/\text{m}^3$ CdCl_2 , respectively, developed lung carcinomas. None of the controls developed lung carcinomas (Takenaka et al., 1993). Another study found that Wistar rats given an oral dose of CdCl_2 through their diet for 77 weeks developed leukemia, prostate tumors, and testis tumors. Rats were treated with 25, 50, 100 and 200 mg/l CdCl_2 . Only rats treated with 50 mg/l CdCl_2 displayed a significant increase in prostatic hyperplasias and adenomas compared to the control. Testicular tumors were only significantly elevated in rats exposed to 200 mg/l cadmium, while large granular lymphocyte leukemia incidence was significantly higher in the groups exposed to 50 and 100 mg/l cadmium (Waalkes and Rehm, 1992). Glasser et al. investigated the ability of various cadmium species to induce lung cancer. The study exposed Wistar rats via inhalation to 0–900 $\mu\text{g}/\text{m}^3$ CdCl_2 (30 and 90 $\mu\text{g}/\text{m}^3$), cadmium sulfate (CdSO_4) (90 $\mu\text{g}/\text{m}^3$) and cadmium sulfide (CdS) (90, 270, 810 and 2430 $\mu\text{g}/\text{m}^3$) for 40 h/wk for 18 months. All species and doses of cadmium increased lung tumor incidence significantly compared to the control (Glaser U, 1990).

In vitro studies have suggested that cadmium may mediate its carcinogenicity via ROS and inhibiting DNA repair mechanisms. BEAS-2b cells treated with 0.125–2 μM cadmium for 2 months became transformed in a dose-dependent manner and the invasive and migratory properties of the cells increased. The study also indicated the involvement of ROS in cadmium-induced carcinogenicity. Transfection with superoxide dismutase (SOD) and catalase (CAT) impeded cadmium's carcinogenicity by reducing the amount of transformed colonies and preventing cell migration and invasion. PI3K, AKT, GSK-3 β , and β -catenin signaling pathways were activated by cadmium exposure but after transfection with SOD and CAT, cadmium-mediated activation of these signaling proteins was inhibited (Son et al., 2012). Cadmium cytotoxicity may be mediated via inhibition of DNA repair mechanisms, i.e. base excision repair, nucleotide excision repair, mismatch repair, and the elimination of 7,8-dihydro-8-oxoguanine. Cadmium has shown to be comutagenic with UV. Cadmium interferes with nucleotide excision repair by interfering with the removal of thymine dimers after UV irradiation (Hartwig and Schwerdtle, 2002).

C. DNA Methylation and Cadmium-Induced Carcinogenesis

i. Hypermethylation: Similar to nickel and arsenic, cadmium exposure causes hypermethylation of tumor suppressor genes. A study conducted by Benbrahim-Tallaa et al. transformed prostate epithelial cells by exposing them to cadmium for 10 weeks. The cadmium-transformed cells overexpressed DNMT3b and displayed an increase in global 5mC and a decreased expression of tumor suppressor genes *RASSF1A* and *P16*. DNMT3b overexpression was accompanied by an increase in DNMT activity. The inactivation of *RASSF1A* and *P16* occurred via hypermethylation of their promoters. Inactivation of DNMT1 reversed the cadmium-induced hypermethylation indicating that DNMT1 is necessary for the maintenance of these effects (Benbrahim-Tallaa et al., 2007). The cadmium-induced hypermethylation and DNMT3b overexpression has potential for clinical use as biomarkers for cadmium-induced prostate cancer, however further research is needed to establish these findings as valid biomarkers.

Another study using HLF cells (Human embryo Lung Fibroblasts) coincided with the results observed by Benbrahim-Tallaa et al. When the cells were treated with 1.5 μM cadmium for 2

months, genomic DNA methylation levels and DNMT activity increased in a dose-dependent manner. mRNA levels of DNMT1, DNMT3a, and DNMT3b were also up-regulated. Cadmium induced cell proliferation by increasing the G0/G1- phase cell population and increasing the population of cells in S-phase (Jiang et al., 2008).

A recent study using a pig Robertsonian translocation model, which is a cross between a wild boar and a domestic pig, examined the effects of cadmium on aneuploidy and global DNA methylation. Cells derived from the pig hybrid were treated with different doses of cadmium sulfate (1 μ M and 5 μ M) for 72 h. A cadmium-mediated increase in aneuploidy was observed along with a global increase in 5mC for both doses as detected by HPLC and immunostaining. Cadmium-induced aneuploidy may be mediated by global DNA hypermethylation and this may be one mechanism of cadmium-induced carcinogenesis (Inglot et al., 2012).

ii. Hypomethylation: A study by Hossein et al. examined the effects of a low-level cadmium exposure in Argentinean women on global DNA methylation. The low-level of urinary cadmium measured in the women reflected the women's lifelong exposure. CG methylation of LINE1 was measured as a surrogate for global DNA methylation. The study found that cadmium exposure was inversely associated with LINE1 methylation and DNMT3b expression. Loss of LINE1 methylation is a common epigenetic event in malignancies and may play a role in cadmium-induced carcinogenesis (Hossain et al., 2012).

Cadmium has been shown to cause cell proliferation in different types of cancer (Fang et al., 2002, Johnson et al., 2003). Huang et al. has reported on the role of DNA methylation in cadmium-stimulated cell proliferation in a chronic myelogenous leukemia cell line (K562 cells). K562 cells were treated with 2.0 μ M CdCl₂ for 24 and 48 hours. Cadmium caused an increase in cellular proliferation, as well as an increase in ROS, DNA damage, and DNA hypomethylation. When the ROS and DNA damage levels were decreased by pre-treatment with N-acetylcysteine, the cadmium-stimulated cell proliferation was not suppressed. Methionine can inhibit hypomethylation caused by many carcinogens. When the cells were pre-treated with methionine, they showed a suppressed level of proliferation and an elevated level of methylation. The study suggested that cadmium-stimulated cell proliferation may be mediated through DNA hypomethylation (Huang et al., 2008).

Several studies have demonstrated that the length of cadmium exposure affects DNA methylation levels. A study by Takiguchi et al. observed that rat liver epithelial cells treated with 2.5 μ M cadmium for 1 week displayed a decrease in genomic DNA methylation, however this same cell line displayed a significant increase in genomic DNA methylation after being treated with the same dose for 10 weeks. DNMT activity was reduced in these cells after the short-term exposure, while the long-term exposure increased DNMT activity (Takiguchi et al., 2003). Another study using HLF cells (Human embryo Lung Fibroblasts) coincided with these results. When the cells were treated with 1.5 μ M cadmium for 2 months, genomic DNA methylation levels and DNMT activity increased (Jiang et al., 2008).

D. Discussion—Similar to the other metals discussed so far, cadmium-induced carcinogenesis involves both hyper- and hypomethylation events. Based on the studies presented above, there seems to be a correlation between length of cadmium exposure, methylation status, and DNMT levels. Table 4 summarizes these findings. It seems that a long-term exposure leads to hypermethylation, whereas a short-term exposure results in hypomethylation. This seems to be the case for the studies presented with the exception of two, Inglot et al. and Hossein et al. The findings by Inglot et al. are unexplained and do not coincide with the results from some of the other studies. However, the increase in methylation reported by this study was not as large as compared to other studies. Variables

such as experimental design, cell type, cadmium compound and 5mC detection methods play a role in the outcomes of the studies. The investigation by Hossein et al. examined lifelong cadmium exposure at very low concentrations- median concentrations in blood and urine were 0.36 and 0.23 $\mu\text{g/l}$, respectively. Such low human exposure may not generate similar results as high exposures using cell lines described in the other studies discussed.

DNMT expression and activity seem to correlate with methylation levels. Hypermethylation induced by a long-term treatment of cadmium is usually accompanied by overexpression of DNMTs and increased DNMT activity, while hypomethylation induced by a short-term treatment of cadmium is correlated with lower DNMT levels. Further investigations should examine the methylation status of the DNMT genes after cadmium exposure. If DNMT expression is modulated by promoter methylation during cadmium carcinogenesis, then promoter hypermethylation would occur simultaneously with global hypomethylation and vice versa. This outcome would not be surprising considering the complex mechanisms that underlie metal-induced carcinogenesis.

The investigation by Takiguchi et al. strengthens the long-term/hypermethylation and short-term/hypomethylation hypothesis the most. This study controls all variables between experiments (cell type, dose, etc.) except length of exposure. Further research investigating this hypothesis should consider involving a wide range of exposure lengths (24h, 72h, 1 wk, 1 mo, 2 mo, etc.) while keeping all other variables the same. DNMT expression and activity levels should be assessed at each time point. This experimental design would allow the investigator to observe the transition from hypomethylation to hypermethylation while providing some insight into the mechanisms underlying cadmium-induced methylation alterations.

While epigenetic alterations seem to be the predominant mechanism of cadmium-induced carcinogenesis, other mechanisms may also play a role in the process. Inhibition of DNA repair, interference of gene regulation and signal transduction, interference with antioxidant enzymes, and disruption of E-cadherin-mediated cell adhesion also characterize the carcinogenic effects of cadmium compounds (Waisberg et al., 2003). These processes are probably not isolated but overlap, and should be considered in future investigations aiming to examine cadmium-induced methylation alterations.

Chromium (VI)

A. Chromium (VI) Exposure—Hexavalent chromium (Cr (VI)) is a well-known human and animal carcinogen (Schnedl et al., 1975) with both environmental and occupational exposures. People are environmentally exposed to Cr (VI) by drinking chromium-contaminated water or through the use of products containing chromium. The color, malleability, and tensile strength of Cr (VI) compounds make it useful in industrial settings such as chromate plating and chromate pigment production. Occupational inhalation exposure to Cr (VI) is associated with lung cancer incidence in epidemiological studies (Gibb et al., 2000). Cr (VI) is the most toxic species of chromium since it is able to enter the cells via anion transport channels (Andrew M. Standeven, 1989). Once in the cell, it ultimately gets reduced to trivalent chromium (Cr (III)) by various cellular reducing agents such as glutathione and ascorbic acid. Cr (III) and reactive intermediates formed during its generation- Cr (IV), Cr (V) and reactive oxygen species- induces DNA damage and chromium-mediated DNA adducts (Lachner et al., 2001, Costa and Klein, 2006). Chromium has been investigated more than any other metal with respect to mutagenicity and carcinogenicity. Although many of the cellular effects of chromium can cause genotoxic stress, recently chromium has been shown to cause toxic and carcinogenic effects through epigenetic mechanisms.

B. Human, Animal and *In Vitro* Studies—In this section, we will discuss some of the investigations that characterize the carcinogenicity of Cr (VI). Table 5 summarizes the human, animal and *in vitro* studies discussed here.

Cr (VI) has been associated with cancer in various human studies. A recent study investigated an association between Cr (VI) and cancer incidence and mortality in Korean cement workers exposed to cement dust, which contains varying levels of Cr (VI). The study examined cancer mortality for 15 years and cancer incidence for 8 years in a cohort of male workers in 6 Portland cement factories in Korea. Each cohort included around 5,000 subjects. The most significant finding in the study was an increased incidence of stomach cancer in production workers. Besides stomach cancer, Cr (VI) in cement dust has been linked to occupational skin diseases, decreased lung function, and increased chronic obstructive pulmonary disease prevalence. Cr (VI) exposure via cement dust may affect other individuals besides cement workers, such as construction workers and people occupying houses/buildings built with cement (Koh et al., 2011).

Many epidemiological studies have found a link between Cr (VI) exposure and lung cancer. A recent study by Halasova et al. examined 64 Cr (VI)-exposed workers and 104 controls diagnosed with lung cancer and compared age at onset of lung cancer and incidence of lung cancer types. The study found that Cr (VI)-exposed workers developed lung cancer 3.51 years earlier than unexposed controls. Also, a higher percentage (8.7% higher) of small cell lung carcinoma was found in Cr (VI)-exposed workers compared to unexposed individual (Halasova et al., 2009). A study conducted by Birk et. al. examined a cohort of about 1000 German chromate production workers and found an association between occupational Cr (VI) exposure and lung cancer. About 26% of the subjects displayed chromium levels of >40 µg/l in urine samples and lung cancer risk was elevated in the high exposure group containing >200 µg/l chromium in urine samples (Birk et al., 2006). Further studies should examine Cr (VI) levels in blood. Urine only gives some indication of Cr (VI) exposure because the compound is reduced to Cr (III) by the time it is excreted in urine.

Inhalation of Cr (VI) is widely considered to be carcinogenic; however, the carcinogenicity of Cr (VI) by ingestion has not been studied as much. An ecological study by Linos et al. investigated cancer mortality in a region of Greece, the Oinofita municipality, where the public water has been contaminated with Cr (VI). The level of Cr (VI) in the public water supply of this region has been measured by different entities from 2007–2010. Concentrations ranged from 8 to 156 µg/l. EPA regulations allow up to 100 µg/l of Cr (VI) in US drinking-water. The study examined a cohort of 5,842 individuals in the Oinofita municipality for 10 years and found that the standard mortality ratios for primary liver cancer, kidney cancer, and lung cancer were significantly high. The elevated cancer mortality in the Oinofita municipality supports the hypothesis that Cr (VI) is carcinogenic via oral exposure pathways (Linos et al., 2011).

Animal bioassay studies have also characterized the carcinogenicity of Cr (VI). A study by Stout et al. investigated the carcinogenic effects of an oral exposure to Cr (VI) in F344/N rats and B6C3F1 mice. Male and female rats were exposed to 14.3, 57.3, 172 or 516 mg/l sodium dichromate dihydrate (SDD) for 2 years. Only rats exposed to the highest dose (516 mg/l) developed significantly more oral neoplasms than control mice. Male and female mice were exposed to 14.3, 28.6, 85.7 or 257.4 mg/l SDD for 2 years. Only mice exposed to the highest dose (257.4 mg/l) developed significantly more intestinal neoplasms than control mice (Stout et al., 2009). Davidson et. al. found that Cr (VI) acts as a cocarcinogen with UV. The group exposed CRL: SK1-hrBR hairless mice to UV alone (1.2 kJ/m²), potassium chromate alone (0.5, 2.5, and 5 mg/l) or UV +potassium chromate for 6 months. The study found that an oral dose of Cr (VI) did not cause skin tumors in mice but when co-exposed

with UV, skin tumor formation significantly increased compared to the control. The increased tumor formation in mice co-exposed to UV and the lowest dose of chromate (0.5 mg/l) was not significant. The co-exposure group also developed significantly more tumors than the group exposed to UV alone (Davidson et al., 2004).

An early study reporting on the mutagenic effects of Cr (VI) compounds *in vitro* was performed by Biedermann et al. The group found that chromium (VI) compounds (lead chromate, potassium dichromate, calcium dichromate) induce cytotoxicity, induce a mutation that causes 6-thioguanine resistance, and induces anchorage independent transformation in human fibroblasts (Kim A. Biedermann, 1987, Biedermann, 1990).

A recent investigation examining the altered gene expression profile of BEAS-2b cells transformed by Cr (VI) gives some insight into the carcinogenic mechanisms of this metal. Cells were treated with 0.25 and 0.5 μ M potassium chromate for 1 month and formed colonies in soft agar. Gene expression analysis was performed on cell lines extracted from soft agar. The genes associated with cell to matrix adhesion were significantly down-regulated in Cr (VI) transformed cells, including many genes involved in focal adhesion. The study also demonstrated a dramatic increase in expression of genes associated with desmosome complex in Cr (VI) transformed cells. Desmosome is an intracellular junction mediating the interaction between adjacent epithelial cells. The level of cyclin D1, an important cyclin expressed in early G1 phase and required for cell cycle progression, was increased in Cr (VI) transformed cells. Dysregulation of cyclin D1 was frequently found in the early stage of tumorigenesis in many different cancers, and has been reported at high levels in chromate-induced lung cancers. TGF β is an important negative regulator of lung epithelial cells, and loss of TGF β signaling is an early event that contributes to cell growth. Cr (VI) transformed cells exhibited significantly reduced levels of TGF β 2 and TGF β R2, and were able to escape from TGF β induced growth inhibition. These results suggested that Cr (VI) may have promoted tumor cell growth by stimulating proliferation-associated genes and inhibiting anti-proliferation genes (Sun et al., 2011).

It is evident in the above studies that Cr (VI) exposure should be a major concern for human health. Cancer is the main endpoint of concern in regards to Cr (VI) exposure. Although Cr (VI) is a known mutagen, it exerts its carcinogenicity largely through epigenetic mechanisms so it is important that close attention is devoted to research that aims to unearth epigenetic mechanisms observed in Cr (VI)-induced cancers.

C. DNA Methylation and Cr (VI)-induced Carcinogenicity

i. Hypermethylation: The first evidence that Cr (VI) could aberrantly induce DNA methylation was shown in G12 cells, a transgenic mammalian cell line that contained the bacterial *gpt* reporter gene. G12 cells were treated with 20, 30, 40 and 50 μ M potassium dichromate for 2 h. Cr (VI) induced at least partial DNA promoter methylation at each dose and silenced the *gpt* gene in these cells (Klein et al., 2002). Potassium dichromate was also shown to alter the cytosine methylation status in *Brassica napus* L. plants. The chemical caused an increase in the genome-wide methylation status of CCGG-sequences in a dose-dependent manner (Labra et al., 2004).

A number of studies have compared DNA methylation levels in lung cancer from chromate-exposed workers versus lung cancer from non-exposed workers. Chromate exposure was associated with the hypermethylation of certain genes involved in cell cycle control and DNA repair.

Chromate lung cancer frequently contains microsatellite instability (MSI) and it is often associated with the loss of expression of *MLH1*, a core mismatch repair (MMR) protein.

Takahashi et al. examined tumors taken from lung cancer patients exposed to chromate and lung cancer patients unexposed to chromate. The study found that repression of *MLH1* was higher in chromate-exposed patients than unexposed patients. After analysis by combined bisulfite restriction analysis (COBRA) method, which observes methylation status of a genomic region, it was found that *MLH1* repression was due to hypermethylation of the promoter region. The frequency of replication error in chromate lung cancer is very high. In chromate lung cancer, the repression rate of *MLH1* was 90% in the group with the highest amount of replication error. This group also contained the highest amount of loci (3 or more) displaying MSI (Takahashi et al., 2005).

A similar study conducted by the same group investigated the methylation status of the *P16* gene by methylation-specific PCR (MSP) in 30 chromate lung cancers and 38 non-chromate lung cancers. 7% more chromate lung cancers displayed *P16* methylation than non-chromate lung cancers. Almost all chromate lung cancer patients who had tumors that displayed *P16* methylation had experienced more than 15 years of chromate exposure. 86% of chromate lung cancers with *P16* methylation also showed repression of the p16 protein (Kondo et al., 2006).

Keeping with the same theme as the previous studies, Ali et al. investigated promoter methylation of *MLH1* and *APC* (adenomatosis polyposis) in 36 chromate lung cancers and 25 non-chromate lung cancers. After analysis using a nested-MSP method, the study found that there was more methylation in *MLH1* and *APC* genes in chromate lung cancers than non-chromate lung cancers. 86% and 28% of chromate lung cancers displayed hypermethylation for *APC* and *MLH1* genes, respectively. Non-chromate lung cancers displayed hypermethylation of *APC* for 44% of subjects and no subjects contained *MLH1* hypermethylation. The overall methylation status was also higher in chromate lung cancers than non-chromate lung cancers (Ali et al., 2011).

ii. Hypomethylation: Although Cr (III) is thought to be largely nontoxic due to its inability to enter cells, a study has demonstrated hypomethylation in germ cells after Cr (III) exposure. Male mice were treated with Cr (III) chloride for 2 weeks and DNA methylation levels in sperm cells were examined. It was found that two regions of the 45S rRNA gene were hypomethylated. Inheritance of these effects was not tested in the offspring (Cheng et al., 2004).

D. Discussion—The evidence presented above suggests that one mechanism of Cr (VI)-induced carcinogenesis involves the perturbation of methylation levels. Cr (VI) is more associated with hypermethylation events than hypomethylation events. The strongest evidence for chromium's ability to perturb the methylome is presented in studies involving lung cancer patients exposed to chromate. Promoter hypermethylation of *P16*, *MLH1*, and *APC* have all shown to be associated with Cr (VI)-induced carcinogenesis. The mechanism that underlies these hypermethylation events has yet to be brought to light. The influence of ROS on Cr (VI)-induced methylation alterations should be considered in future research. It would not be surprising if ROS was a mediator of these hypermethylation events since many carcinogenic and toxic outcomes induced by metals involves ROS and Cr (VI) reduction is known to create ROS, as well as many other reactive intermediates.

The investigation conducted by Takahashi et al. provides a possible mechanism of Cr (VI)-induced carcinogenesis. The study found that *MLH1* was repressed by promoter hypermethylation in chromate-exposed lung cancers. The repression of a DNA repair protein would allow the cell to take on a mutator phenotype which would potentiate carcinogenesis by accelerating the activating mutations of oncogenes and inactivating mutations of tumor suppressor genes. Microsatellite instability is the expansion or

contraction in tandem repeated DNA sequences of 1 to 6 nucleotides and is often caused by defective MMR. Chromate lung cancers with repressed *MLH1* were associated with an increased amount of loci displaying MSI. This mechanism of carcinogenesis is similar to what is observed in hereditary nonpolyposis colorectal cancer (HNPCC). Promoter hypermethylation of *MLH1* has been established as a cost effective biomarker for HNPCC and should be considered as a possible biomarker for chromate-induced cancers. In order to validate its use as a biomarker, further investigations should aim to examine the association between *MLH1* hypermethylation and Cr (VI) exposure.

Besides *MLH1* repression, chromium has also been shown to inhibit other DNA repair mechanisms. *OGG1*, the glycosylase that repairs 8-oxo-guanine, was down-regulated by sodium dichromate in A549 cells (human lung cancer cells) (Hodges and Chipman, 2002). Rats exposed to chromate by inhalation contained lung tissue that displayed decreased expression of *OGG1* (Maeng et al., 2003). By inhibiting *OGG1*, ROS-induced damage generated by Cr (VI) goes unrepaired. It seems that Cr (VI) has the tendency to inhibit critical genes involved in protecting genomic integrity in order to exert its toxic and carcinogenic effects. The molecular mechanisms underlying chromium's inhibition of DNA repair are still unknown. Future studies should further investigate chromium's effects on other DNA repair mechanisms involving oxidative damage since it is possible that inhibition of repair proteins may constitute a principle mechanism in Cr (VI)-induced carcinogenesis. Chromium is a known mutagen (Biedermann KA, 1987, Biedermann, 1990), and it is likely that chromium-induced carcinogenesis is a combination of genetic and epigenetic events.

VI. CONCLUSION

DNA methylation plays an intricate role in the regulation of gene expression and events that compromise the integrity of the methylome have the potential to contribute to disease development. This review has discussed the basic mechanics of DNA methylation and its disruption after metal exposure. 5mC is a reversible and inheritable modification formed by writers (DNMTs), recognized by readers (MDPs), and presumably removed by erasers (Tet proteins). DNA methylation is a regulatory mechanism that triggers a path of events leading to chromatin condensation and gene silencing.

Cancer cells display a unique landscape of DNA methylation that is induced by environmental agents such as metals. The evidence described above summarizes the effects metals have on the local level to the genomic level of DNA. While investigations continue to identify DNA methylation perturbations induced by carcinogenic metals, the mechanisms behind the paradoxical effects metals have on the methylome calls for further investigation. Future studies that better illustrate the methylome changes induced by metals are imperative to understand the epigenetic mechanisms that underlie metal-induced carcinogenesis. It is essential for future research to be conducted in a manner that discerns whether metal-induced DNA methylation alterations occur due to direct effects of metals or occur just as secondary effects of tumorigenesis. Model systems must be designed to investigate DNA methylation alterations with higher relevance to humans. While much of the research findings in this field hold potential implications as biomarkers for cancer detection, further investigations, including human studies, are required to establish these findings as valid biomarkers. Also, the DNA methylation changes that are induced by metals may not be specific to metals; other environmental epimutagens may cause similar methylome alterations that resemble cancer (Zhao and Bu, 2012, Lim and Song, 2012).

Repression of DNA repair genes allows the cell to acquire mutations at an accelerated rate. Due to substantial evidence indicating the ability of metals to hypermethylate and silence DNA repair genes, further investigations should examine these critical genes. Research

groups who have tissues and samples stored from previous studies should consider using their specimens for such analysis. In order to establish a promoter hypermethylation of a certain gene as a hallmark of metal exposure, more evidence needs to be provided. Future research should investigate DNA repair genes that have been previously found to be hypermethylated after exposure to a certain metal compound, e.g. *MGMT* and nickel compounds, *MLH1* and chromium (VI) compounds.

Advances in epigenomics brought about by methylation-sensitive deep sequencing and microarrays will increase the accuracy and depth of research performed in this field. With the rise of personalized medicine, characteristics of the cancer methylome will be harnessed to develop drugs to better treat and prevent cancer. Since cancer cells display a variety of DNA methylation alterations- both hyper- hypomethylation events-, it is important to consider the detrimental modulations brought about by current drugs that attempt to reverse the methylation profile of a cancer cell. For example, broad drugs such as DNA methylation inhibitors that attempt to reverse the methylation state of hypermethylated tumor suppressor genes may actually promote disease progression since cancer is also characterized by hypomethylation events. Drugs with a specific target must be developed in order to have a meaningful impact in cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- ABALEA V, CILLARD J, DUBOS MP, SERGENT O, CILLARD P, MOREL I. Repair of iron-induced DNA oxidation by the flavonoid myricetin in primary rat hepatocyte cultures. *Free Radic Biol Med.* 1999; 26:1457–66. [PubMed: 10401609]
- ADAMS SV, PASSARELLI MN, NEWCOMB PA. Cadmium exposure and cancer mortality in the Third National Health and Nutrition Examination Survey cohort. *Occup Environ Med.* 2012; 69:153–6. [PubMed: 22068173]
- AGUILERA O, FERNANDEZ AF, MUNOZ A, FRAGA MF. Epigenetics and environment: a complex relationship. *J Appl Physiol.* 2010; 109:243–51. [PubMed: 20378707]
- ANDREW M, STANDEVEN KEW. Chromium(VI) Toxicity: Uptake, Reduction, and DNA Damage. *International Journal of Toxicology.* 1989; 8:1275–1283.
- ANWAR SL, KRECH T, HASEMEIER B, SCHIPPER E, SCHWEITZER N, VOGEL A, KREIPE H, LEHMANN U. Loss of imprinting and allelic switching at the DLK1-MEG3 locus in human hepatocellular carcinoma. *PLoS One.* 2012; 7:e49462. [PubMed: 23145177]
- APOSHIAN HV. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu Rev Pharmacol Toxicol.* 1997; 37:397–419. [PubMed: 9131259]
- ARITA A, COSTA M. Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium. *Metallomics.* 2009; 1:222–8. [PubMed: 20461219]
- ARITA A, NIU J, QU Q, ZHAO N, RUAN Y, NADAS A, CHERVONA Y, WU F, SUN H, HAYES RB, COSTA M. Global levels of histone modifications in peripheral blood mononuclear cells of subjects with exposure to nickel. *Environ Health Perspect.* 2012; 120:198–203. [PubMed: 22024396]
- BENBRAHIM-TALLAA L, WATERLAND RA, DILL AL, WEBBER MM, WAALKES MP. Tumor suppressor gene inactivation during cadmium-induced malignant transformation of human prostate cells correlates with overexpression of de novo DNA methyltransferase. *Environ Health Perspect.* 2007; 115:1454–9. [PubMed: 17938735]
- BENBRAHIM-TALLAA L, WATERLAND RA, STYBLO M, ACHANZAR WE, WEBBER MM, WAALKES MP. Molecular events associated with arsenic-induced malignant transformation of human prostatic epithelial cells: aberrant genomic DNA methylation and K-ras oncogene activation. *Toxicol Appl Pharmacol.* 2005; 206:288–98. [PubMed: 16039940]

- BETTSTETTER M, WOENCKHAUS M, WILD PJ, RUMMELE P, BLASZYK H, HARTMANN A, HOFSTADTER F, DIETMAIER W. Elevated nuclear maspin expression is associated with microsatellite instability and high tumour grade in colorectal cancer. *J Pathol.* 2005; 205:606–14. [PubMed: 15714592]
- BIEDERMANN KALJ. Induction of anchorage independence in human diploid foreskin fibroblasts by carcinogenic metal salts. *Cancer Research.* 1987; 47:3815–3823. [PubMed: 3594439]
- BIEDERMANN KAAL JR. Role of Valence State and Solubility of Chromium Compounds on Induction of Cytotoxicity, Mutagenesis, and Anchorage Independence in Diploid Human Fibroblasts. *Cancer Research.* 1990; 50:7835–7842. [PubMed: 2253225]
- BIRK T, MUNDT KA, DELL LD, LUIPPOLD RS, MIKSCH L, STEINMANN-STEINER-HALDENSTAETT W, MUNDT DJ. Lung cancer mortality in the German chromate industry, 1958 to 1998. *J Occup Environ Med.* 2006; 48:426–33. [PubMed: 16607199]
- BOLTZE C, SCHNEIDER-STOCK R, QUEDNOW C, HINZE R, MAWRIN C, HRIBASCHEK A, ROESSNER A, HOANG-VU C. Silencing of the maspin gene by promoter hypermethylation in thyroid cancer. *Int J Mol Med.* 2003; 12:479–84. [PubMed: 12964023]
- BOSTICK M, KIM JK, ESTEVE PO, CLARK A, PRADHAN S, JACOBSEN SE. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science.* 2007; 317:1760–4. [PubMed: 17673620]
- BRANDEIS M, FRANK D, KESHET I, SIEGFRIED Z, MENDELSON M, NEMES A, TEMPER V, RAZIN A, CEDAR H. Sp1 elements protect a CpG island from de novo methylation. *Nature.* 1994; 371:435–8. [PubMed: 8090226]
- CANNUYER J, LORIOT A, PARVIZI GK, DE SMET C. Epigenetic Hierarchy within the MAGEA1 Cancer-Germline Gene: Promoter DNA Methylation Dictates Local Histone Modifications. *PLoS One.* 2013; 8:e58743. [PubMed: 23472218]
- CENTENO JA, MULLICK FG, MARTINEZ L, PAGE NP, GIBB H, LONGFELLOW D, THOMPSON C, LADICH ER. Pathology related to chronic arsenic exposure. *Environ Health Perspect.* 2002; 110(Suppl 5):883–6. [PubMed: 12426152]
- CHANDA S, DASGUPTA UB, GUHAMAZUMDER D, GUPTA M, CHAUDHURI U, LAHIRI S, DAS S, GHOSH N, CHATTERJEE D. DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. *Toxicol Sci.* 2006; 89:431–7. [PubMed: 16251483]
- CHEN CJ, CHUANG YC, LIN TM, WU HY. Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: high-arsenic artesian well water and cancers. *Cancer Res.* 1985; 45:5895–9. [PubMed: 4053060]
- CHEN H, GIRI NC, ZHANG R, YAMANE K, ZHANG Y, MARONEY M, COSTA M. Nickel ions inhibit histone demethylase JMJD1A and DNA repair enzyme ABH2 by replacing the ferrous iron in the catalytic centers. *J Biol Chem.* 2010a; 285:7374–83. [PubMed: 20042601]
- CHEN H, KLUZ T, ZHANG R, COSTA M. Hypoxia and nickel inhibit histone demethylase JMJD1A and repress Spry2 expression in human bronchial epithelial BEAS-2B cells. *Carcinogenesis.* 2010b; 31:2136–44. [PubMed: 20881000]
- 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; 25:1779–86. [PubMed: 15073043]
- CHEN PH, LAN CC, CHIOU MH, HSIEH MC, CHEN GS. Effects of arsenic and UVB on normal human cultured keratinocytes: impact on apoptosis and implication on photocarcinogenesis. *Chem Res Toxicol.* 2005; 18:139–44. [PubMed: 15720117]
- CHENG RY, HOCKMAN T, CRAWFORD E, ANDERSON LM, SHIAO YH. Epigenetic and gene expression changes related to transgenerational carcinogenesis. *Mol Carcinog.* 2004; 40:1–11. [PubMed: 15108325]
- CHERVONA Y, COSTA M. The control of histone methylation and gene expression by oxidative stress, hypoxia, and metals. *Free Radic Biol Med.* 2012; 53:1041–7. [PubMed: 22841757]
- CHERVONA Y, HALL MN, ARITA A, WU F, SUN H, TSENG HC, ALI E, UDDIN MN, LIU X, ZORODDU MA, GAMBLE MV, COSTA M. Associations between arsenic exposure and global

- posttranslational histone modifications among adults in Bangladesh. *Cancer Epidemiol Biomarkers Prev.* 2012; 21:2252–60. [PubMed: 23064002]
- CIMMINO A, CALIN GA, FABBRI M, IORIO MV, FERRACIN M, SHIMIZU M, WOJCIK SE, AQEILAN RI, ZUPO S, DONO M, RASSENTI L, ALDER H, VOLINIA S, LIU CG, KIPPS TJ, NEGRINI M, CROCE CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A.* 2005; 102:13944–9. [PubMed: 16166262]
- CONWAY K, COSTA M. Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. *Cancer Res.* 1989; 49:6032–8. [PubMed: 2790817]
- COSTA M, KLEIN CB. Toxicity and carcinogenicity of chromium compounds in humans. *Crit Rev Toxicol.* 2006; 36:155–63. [PubMed: 16736941]
- CRUZ MJ, COSTA R, MARQUILLES E, MORELL F, MUNOZ X. Occupational asthma caused by chromium and nickel. *Arch Bronconeumol.* 2006; 42:302–6. [PubMed: 16827980]
- 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.* 2006a; 91:372–81. [PubMed: 16543296]
- CUI X, WAKAI T, SHIRAI Y, YOKOYAMA N, HATAKEYAMA K, HIRANO S. Arsenic trioxide inhibits DNA methyltransferase and restores methylation-silenced genes in human liver cancer cells. *Hum Pathol.* 2006b; 37:298–311. [PubMed: 16613325]
- DAVIDSON T, KLUZ T, BURNS F, ROSSMAN T, ZHANG Q, UDDIN A, NADAS A, COSTA M. Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. *Toxicol Appl Pharmacol.* 2004; 196:431–7. [PubMed: 15094314]
- DE SMET C, LORIOT A. DNA hypomethylation and activation of germline-specific genes in cancer. *Adv Exp Med Biol.* 2013; 754:149–66. [PubMed: 22956500]
- DOLINOY DC, WEIDMAN JR, WATERLAND RA, JIRTLE RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect.* 2006; 114:567–72. [PubMed: 16581547]
- DRUWE IL, VAILLANCOURT RR. Influence of arsenate and arsenite on signal transduction pathways: an update. *Arch Toxicol.* 2010; 84:585–96. [PubMed: 20502880]
- DUNNICK JK, ELWELL MR, RADOVSKY AE, BENSON JM, HAHN FF, NIKULA KJ, BARR EB, HOBBS CH. Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate chronic exposures in the lung. *Cancer Res.* 1995; 55:5251–6. [PubMed: 7585584]
- EBERT F, WEISS A, BULTEMEYER M, HAMANN I, HARTWIG A, SCHWERDTLE T. Arsenicals affect base excision repair by several mechanisms. *Mutat Res.* 2011; 715:32–41. [PubMed: 21782832]
- EDEN A, GAUDET F, WAGHMARE A, JAENISCH R. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science.* 2003; 300:455. [PubMed: 12702868]
- ESTELLER M. Epigenetics in cancer. *N Engl J Med.* 2008; 358:1148–59. [PubMed: 18337604]
- FANG MZ, MAR W, CHO MH. Cadmium affects genes involved in growth regulation during two-stage transformation of Balb/3T3 cells. *Toxicology.* 2002; 177:253–65. [PubMed: 12135628]
- FAZZARI MJ, GREALLY JM. Epigenomics: beyond CpG islands. *Nat Rev Genet.* 2004; 5:446–55. [PubMed: 15153997]
- FREITAS M, BARCELLOS-DE-SOUZA P, BARJA-FIDALGO C, FERNANDES E. Nickel induces apoptosis in human neutrophils. *Biometals.* 2012
- FUKS F, HURD PJ, WOLF D, NAN X, BIRD AP, KOUZARIDES T. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J Biol Chem.* 2003; 278:4035–40. [PubMed: 12427740]
- GIBB HJ, LEES PS, PINSKY PF, ROONEY BC. Lung cancer among workers in chromium chemical production. *Am J Ind Med.* 2000; 38:115–26. [PubMed: 10893504]
- GLASER UH, OTTO FJ, OLDIGES H. Carcinogenicity and toxicity of four cadmium compounds inhaled by rats. *Toxicol Environ Chem.* 1990; 27:153–162.
- GOKUL G, KHOSLA S. DNA methylation and cancer. *Subcell Biochem.* 2012; 61:597–625. [PubMed: 23150269]

- GOVINDARAJAN B, KLAFTER R, MILLER MS, MANSUR C, MIZESKO M, BAI X, LAMONTAGNE K JR, ARBISER JL. Reactive oxygen-induced carcinogenesis causes hypermethylation of p16(Ink4a) and activation of MAP kinase. *Mol Med*. 2002; 8:1–8. [PubMed: 11984000]
- GUHA MAZUMDER D, DASGUPTA UB. Chronic arsenic toxicity: studies in West Bengal, India. *Kaohsiung J Med Sci*. 2011; 27:360–70. [PubMed: 21914522]
- HALASOVA E, MATAKOVA T, KAVCOVA E, MUSAK L, LETKOVA L, ADAMKOV M, ONDRUSOVA M, BUKOVSKA E, SINGLIAR A. Human lung cancer and hexavalent chromium exposure. *Neuro Endocrinol Lett*. 2009; 30(Suppl 1):182–5. [PubMed: 20027168]
- HANADA N, TAKAHATA T, ZHOU Q, YE X, SUN R, ITOH J, ISHIGURO A, KIJIMA H, MIMURA J, ITOH K, FUKUDA S, SAIJO Y. Methylation of the KEAP1 gene promoter region in human colorectal cancer. *BMC Cancer*. 2012; 12:66. [PubMed: 22325485]
- HARTWIG A, SCHWERDTLE T. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicol Lett*. 2002; 127:47–54. [PubMed: 12052640]
- HENDRICH B, HARDELAND U, NG HH, JIRICNY J, BIRD A. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. *Nature*. 1999; 401:301–4. [PubMed: 10499592]
- 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 U S A*. 1998; 95:6870–5. [PubMed: 9618505]
- HERMANN A, GOYAL R, JELTSCH A. The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites. *J Biol Chem*. 2004; 279:48350–9. [PubMed: 15339928]
- HODGES NJ, CHIPMAN JK. Down-regulation of the DNA-repair endonuclease 8-oxo-guanine DNA glycosylase 1 (hOGG1) by sodium dichromate in cultured human A549 lung carcinoma cells. *Carcinogenesis*. 2002; 23:55–60. [PubMed: 11756223]
- HOSSAIN MB, VAHTER M, CONCHA G, BROBERG K. Low-level environmental cadmium exposure is associated with DNA hypomethylation in argentinean women. *Environ Health Perspect*. 2012; 120:879–84. [PubMed: 22382075]
- HOSTYNEK JJ. Sensitization to nickel: etiology, epidemiology, immune reactions, prevention, and therapy. *Rev Environ Health*. 2006; 21:253–80. [PubMed: 17243350]
- HUANG D, ZHANG Y, QI Y, CHEN C, JI W. Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. *Toxicol Lett*. 2008; 179:43–7. [PubMed: 18482805]
- HUANG X, FRENKEL K, KLEIN CB, COSTA M. Nickel induces increased oxidants in intact cultured mammalian cells as detected by dichlorofluorescein fluorescence. *Toxicol Appl Pharmacol*. 1993; 120:29–36. [PubMed: 8511780]
- HUANG YC, HUNG WC, CHEN WT, YU HS, CHAI CY. Sodium arsenite-induced DAPK promoter hypermethylation and autophagy via ERK1/2 phosphorylation in human uroepithelial cells. *Chem Biol Interact*. 2009; 181:254–62. [PubMed: 19577553]
- INGLOT P, LEWINSKA A, POTOCKI L, OKLEJEWICZ B, TABECKA-LONCZYNSKA A, KOZIOROWSKI M, BUGNO-PONIEWIERSKA M, BARTOSZ G, WNUK M. Cadmium-induced changes in genomic DNA-methylation status increase aneuploidy events in a pig Robertsonian translocation model. *Mutat Res*. 2012
- IWITZKI FSR, EICHHORN U, KAINA B, BEYERSMANN D, HARTWIG A. Nickel(II) inhibits the repair of O6-methylguanine in mammalian cells. *Arch Toxicol*. 1998; 72:681–689. [PubMed: 9879805]
- JENSEN TJ, NOVAK P, EBLIN KE, GANDOLFI AJ, FUTSCHER BW. Epigenetic remodeling during arsenical-induced malignant transformation. *Carcinogenesis*. 2008; 29:1500–8. [PubMed: 18448484]
- JI W, YANG L, YU L, YUAN J, HU D, ZHANG W, YANG J, PANG Y, LI W, LU J, FU J, CHEN J, LIN Z, CHEN W, ZHUANG Z. Epigenetic silencing of O6-methylguanine DNA

- methyltransferase gene in NiS-transformed cells. *Carcinogenesis*. 2008; 29:1267–75. [PubMed: 18204074]
- JIANG G, XU L, SONG S, ZHU C, WU Q, ZHANG L, WU L. Effects of long-term low-dose cadmium exposure on genomic DNA methylation in human embryo lung fibroblast cells. *Toxicology*. 2008; 244:49–55. [PubMed: 18077075]
- JIN H, LOU F, YU L. p15 gene expression in acute lymphoblastic leukemia cell line Molt4 induced by arsenic trioxide. *Zhonghua Xue Ye Xue Za Zhi*. 2001; 22:24–6. [PubMed: 11877046]
- JOHNSON MD, KENNEY N, STOICA A, HILAKIVI-CLARKE L, SINGH B, CHEPKO G, CLARKE R, SHOLLER PF, LIRIO AA, FOSS C, REITER R, TROCK B, PAIK S, MARTIN MB. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat Med*. 2003; 9:1081–4. [PubMed: 12858169]
- JONES PA, BAYLIN SB. The epigenomics of cancer. *Cell*. 2007; 128:683–92. [PubMed: 17320506]
- JONES PA, LIANG G. Rethinking how DNA methylation patterns are maintained. *Nat Rev Genet*. 2009; 10:805–11. [PubMed: 19789556]
- JURKOWSKA RZ, JURKOWSKI TP, JELTSCH A. Structure and function of mammalian DNA methyltransferases. *Chembiochem*. 2011; 12:206–22. [PubMed: 21243710]
- KARGACIN B, KLEIN CB, COSTA M. Mutagenic responses of nickel oxides and nickel sulfides in Chinese hamster V79 cell lines at the xanthine-guanine phosphoribosyl transferase locus. *Mutat Res*. 1993; 300:63–72. [PubMed: 7683771]
- KARPF AR, MATSUI S. Genetic disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. *Cancer Res*. 2005; 65:8635–9. [PubMed: 16204030]
- KIM A, BIEDERMANN AJRL. Induction of Anchorage Independence in Human Diploid Foreskin Fibroblasts by Carcinogenic Metal Salts. *Cancer Research*. 1987;47. [PubMed: 3791220]
- KINNIBURGH DG, KOSMUS W. Arsenic contamination in groundwater: some analytical considerations. *Talanta*. 2002; 58:165–80. [PubMed: 18968743]
- KLEIN CB, COSTA M. DNA methylation, heterochromatin and epigenetic carcinogens. *Mutat Res*. 1997; 386:163–80. [PubMed: 9113117]
- KLEIN CB, SU L, BOWSER D, LESZCZYNSKA J. Chromate-induced epimutations in mammalian cells. *Environ Health Perspect*. 2002; 110(Suppl 5):739–43. [PubMed: 12426123]
- KNOBELOCH LM, ZIEROLD KM, ANDERSON HA. Association of arsenic-contaminated drinking-water with prevalence of skin cancer in Wisconsin's Fox River Valley. *J Health Popul Nutr*. 2006; 24:206–13. [PubMed: 17195561]
- KOH DH, KIM TW, JANG SH, RYU HW. Cancer mortality and incidence in cement industry workers in Korea. *Saf Health Work*. 2011; 2:243–9. [PubMed: 22953208]
- KULIS M, ESTELLER M. DNA methylation and cancer. *Adv Genet*. 2010; 70:27–56. [PubMed: 20920744]
- KURTIO P, PUKKALA E, KAHILIN H, AUVINEN A, PEKKANEN J. Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. *Environ Health Perspect*. 1999; 107:705–10. [PubMed: 10464069]
- LABRA M, GRASSI F, IMAZIO S, DI FABIO T, CITTERIO S, SGORBATI S, AGRADI E. Genetic and DNA-methylation changes induced by potassium dichromate in *Brassica napus* L. *Chemosphere*. 2004; 54:1049–58. [PubMed: 14664833]
- LACHNER M, O'CARROLL D, REA S, MECHTLER K, JENUWEIN T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature*. 2001; 410:116–20. [PubMed: 11242053]
- LEE YW, KLEIN CB, KARGACIN B, SALNIKOW K, KITAHARA J, DOWJAT K, ZHITKOVICH A, CHRISTIE NT, COSTA M. Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol Cell Biol*. 1995; 15:2547–57. [PubMed: 7537850]
- LEONARDI G, VAHTER M, CLEMENS F, GOESSLER W, GURZAU E, HEMMINKI K, HOUGH R, KOPPOVA K, KUMAR R, RUDNAI P, SURDU S, FLETCHER T. Inorganic arsenic and basal cell carcinoma in areas of Hungary, Romania, and Slovakia: a case-control study. *Environ Health Perspect*. 2012; 120:721–6. [PubMed: 22436128]

- LIM U, SONG MA. Dietary and lifestyle factors of DNA methylation. *Methods Mol Biol.* 2012; 863:359–76. [PubMed: 22359306]
- LINOS A, PETRALIAS A, CHRISTOPHI CA, CHRISTOFORIDOU E, KOUROUTOU P, STOLTIDIS M, VELOUDAKI A, TZALA E, MAKRIKIS KC, KARAGAS MR. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece--an ecological study. *Environ Health.* 2011; 10:50. [PubMed: 21609468]
- LIU J, BENBRAHIM-TALLAA L, QIAN X, YU L, XIE Y, BOOS J, QU W, WAALKES MP. Further studies on aberrant gene expression associated with arsenic-induced malignant transformation in rat liver TRL1215 cells. *Toxicol Appl Pharmacol.* 2006; 216:407–15. [PubMed: 16876216]
- MAENG SH, CHUNG HW, YU IJ, KIM HY, LIM CH, KIM KJ, KIM SJ, OOTSUYAMA Y, KASAI H. Changes of 8-OH-dG levels in DNA and its base excision repair activity in rat lungs after inhalation exposure to hexavalent chromium. *Mutat Res.* 2003; 539:109–16. [PubMed: 12948819]
- MAJUMDAR S, CHANDA S, GANGULI B, MAZUMDER DN, LAHIRI S, DASGUPTA UB. Arsenic exposure induces genomic hypermethylation. *Environ Toxicol.* 2010; 25:315–8. [PubMed: 19437452]
- MATEOS S, DOMINGUEZ I, PASTOR N, CANTERO G, CORTES F. The DNA demethylating 5-azaC induces endoreduplication in cultured Chinese hamster cells. *Mutat Res.* 2005; 578:33–42. [PubMed: 16202795]
- MAYER W, NIVELEAU A, WALTER J, FUNDELE R, HAAF T. Demethylation of the zygotic paternal genome. *Nature.* 2000; 403:501–2. [PubMed: 10676950]
- MAZUMDER DN. Effect of chronic intake of arsenic-contaminated water on liver. *Toxicol Appl Pharmacol.* 2005; 206:169–75. [PubMed: 15967205]
- MELIKER NJ JR. Arsenic in drinking water and bladder cancer: review of epidemiological evidence. *Trace Metals and other Contaminants in the Environment.* 2007:551–584.
- MIRANDA TB, JONES PA. DNA methylation: the nuts and bolts of repression. *J Cell Physiol.* 2007; 213:384–90. [PubMed: 17708532]
- MISRA RR, SMITH GT, WAALKES MP. Evaluation of the direct genotoxic potential of cadmium in four different rodent cell lines. *Toxicology.* 1998; 126:103–14. [PubMed: 9620542]
- MIURA TPS, SAKURAMOTO T, LANDOLPH. Morphological and neoplastic transformation of C3H/10T1/2 Cl 8 mouse embryo cells by insoluble carcinogenic nickel compounds. *Environ Mol Mutagen.* 1989; 14:65–78. [PubMed: 2548861]
- MURGATROYD C, WU Y, BOCKMUHL Y, SPENGLER D. The Janus face of DNA methylation in aging. *Aging (Albany NY).* 2010; 2:107–10. [PubMed: 20354272]
- NAN X, NG HH, JOHNSON CA, LAHERTY CD, TURNER BM, EISENMAN RN, BIRD A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature.* 1998; 393:386–9. [PubMed: 9620804]
- NAWROT T, PLUSQUIN M, HOGERVORST J, ROELS HA, CELIS H, THIJS L, VANGRONSVELD J, VAN HECKE E, STAESSEN JA. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. *Lancet Oncol.* 2006; 7:119–26. [PubMed: 16455475]
- NOHARA K, BABA T, MURAI H, KOBAYASHI Y, SUZUKI T, TATEISHI Y, MATSUMOTO M, NISHIMURA N, SANO T. Global DNA methylation in the mouse liver is affected by methyl deficiency and arsenic in a sex-dependent manner. *Arch Toxicol.* 2011; 85:653–61. [PubMed: 20978746]
- NOYER-WEIDNER M, TRAUTNER TA. Methylation of DNA in prokaryotes. *EXS.* 1993; 64:39–108. [PubMed: 8380352]
- OKOJI RS, YU RC, MARONPOT RR, FROINES JR. Sodium arsenite administration via drinking water increases genome-wide and Ha-ras DNA hypomethylation in methyl-deficient C57BL/6J mice. *Carcinogenesis.* 2002; 23:777–85. [PubMed: 12016150]
- OLLER AR, KIRKPATRICK DT, RADOVSKY A, BATES HK. Inhalation carcinogenicity study with nickel metal powder in Wistar rats. *Toxicol Appl Pharmacol.* 2008; 233:262–75. [PubMed: 18822311]

- OOI SK, O'DONNELL AH, BESTOR TH. Mammalian cytosine methylation at a glance. *J Cell Sci.* 2009; 122:2787–91. [PubMed: 19657014]
- OTTOLENGHI AD, HASEMAN JK, PAYNE WW, FALK HL, MACFARLAND HN. Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. *J Natl Cancer Inst.* 1975; 54:1165–72. [PubMed: 165308]
- PARK IS, CHANG X, LOYO M, WU G, CHUANG A, KIM MS, CHAE YK, LYFORD-PIKE S, WESTRA WH, SAUNDERS JR, SIDRANSKY D, PAI SI. Characterization of the methylation patterns in human papillomavirus type 16 viral DNA in head and neck cancers. *Cancer Prev Res (Phila).* 2011; 4:207–17. [PubMed: 21292634]
- PARK JS, JEONG YS, SHIN ST, LEE KK, KANG YK. Dynamic DNA methylation reprogramming: active demethylation and immediate remethylation in the male pronucleus of bovine zygotes. *Dev Dyn.* 2007; 236:2523–33. [PubMed: 17676637]
- PI J, HE Y, BORTNER C, HUANG J, LIU J, ZHOU T, QU W, NORTH SL, KASPRZAK KS, DIWAN BA, CHIGNELL CF, WAALKES MP. Low level, long-term inorganic arsenite exposure causes generalized resistance to apoptosis in cultured human keratinocytes: potential role in skin co-carcinogenesis. *Int J Cancer.* 2005; 116:20–6. [PubMed: 15756686]
- POOL-ZOBEL BL, LOTZMANN N, KNOLL M, KUCHENMEISTER F, LAMBERTZ R, LEUCHT U, SCHRODER HG, SCHMEZER P. Detection of genotoxic effects in human gastric and nasal mucosa cells isolated from biopsy samples. *Environ Mol Mutagen.* 1994; 24:23–45. [PubMed: 7519553]
- QAYYUM S, ARA A, USMANI JA. Effect of nickel and chromium exposure on buccal cells of electroplaters. *Toxicol Ind Health.* 2012; 28:74–82. [PubMed: 21636684]
- RAMSAHOYE BH, BINISZKIEWICZ D, LYKO F, CLARK V, BIRD AP, JAENISCH R. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A.* 2000; 97:5237–42. [PubMed: 10805783]
- RAZIN A, SZYF M, KAFRI T, ROLL M, GILOH H, SCARPA S, CAROTTI D, CANTONI GL. Replacement of 5-methylcytosine by cytosine: a possible mechanism for transient DNA demethylation during differentiation. *Proc Natl Acad Sci U S A.* 1986; 83:2827–31. [PubMed: 3458243]
- ROSSMAN TG, KLEIN CB. Genetic and epigenetic effects of environmental arsenicals. *Metallomics.* 2011; 3:1135–41. [PubMed: 21976018]
- ROUGIER N, BOURC'HIS D, GOMES DM, NIVELEAU A, PLACHOT M, PALDI A, VIEGAS-PEQUIGNOT E. Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev.* 1998; 12:2108–13. [PubMed: 9679055]
- SALNIKOW K, DAVIDSON T, ZHANG Q, CHEN LC, SU W, COSTA M. The involvement of hypoxia-inducible transcription factor-1-dependent pathway in nickel carcinogenesis. *Cancer Res.* 2003; 63:3524–30. [PubMed: 12839937]
- SALNIKOW K, ZHITKOVICH A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem Res Toxicol.* 2008; 21:28–44. [PubMed: 17970581]
- SATARUG S, BAKER JR, URBENJAPOL S, HASWELL-ELKINS M, REILLY PE, WILLIAMS DJ, MOORE MR. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol Lett.* 2003; 137:65–83. [PubMed: 12505433]
- SCHMID M, ZIMMERMANN S, KRUG HF, SURES B. Influence of platinum, palladium and rhodium as compared with cadmium, nickel and chromium on cell viability and oxidative stress in human bronchial epithelial cells. *Environ Int.* 2007; 33:385–90. [PubMed: 17250893]
- SCHNEIDL W, DEV VG, TANTRAHAHI R, MILLER DA, ERLANGER BF, MILLER OJ. 5-Methylcytosine in heterochromatic regions of chromosomes: chimpanzee and gorilla compared to the human. *Chromosoma.* 1975; 52:59–66. [PubMed: 1175458]
- SHARIF J, KOSEKI H. Recruitment of Dnmt1 roles of the SRA protein Np95 (Uhrf1) and other factors. *Prog Mol Biol Transl Sci.* 2011; 101:289–310. [PubMed: 21507355]
- SHEN J, WANIBUCHI H, SALIM EI, WEI M, KINOSHITA A, YOSHIDA K, ENDO G, FUKUSHIMA S. Liver tumorigenicity of trimethylarsine oxide in male Fischer 344 rats--

- association with oxidative DNA damage and enhanced cell proliferation. *Carcinogenesis*. 2003; 24:1827–35. [PubMed: 12919961]
- SHEN SF, SHEN JZ, FU HY, WU DS, XU CB, ZHU YF. Mechanism of As(2)O(3) on hdp1 gene demethylation in Jurkat cell line. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2010; 18:1484–8. [PubMed: 21176356]
- STOUT MD, HERBERT RA, KISSLING GE, COLLINS BJ, TRAVLOS GS, WITT KL, MELNICK RL, ABDO KM, MALARKEY DE, HOOTH MJ. Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. *Environ Health Perspect*. 2009; 117:716–22. [PubMed: 19479012]
- STYBLO M, DROBNA Z, JASPERS I, LIN S, THOMAS DJ. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ Health Perspect*. 2002; 110(Suppl 5): 767–71. [PubMed: 12426129]
- SUN H, CLANCY HA, KLUZ T, ZAVADIL J, COSTA M. Comparison of gene expression profiles in chromate transformed BEAS-2B cells. *PLoS One*. 2011; 6:e17982. [PubMed: 21437242]
- SUNDERMAN, FW, JR. Carcinogenicity of nickel compounds in animals. *IARC Sci Publ*; 1984. p. 127–42.
- TAKIGUCHI M, ACHANZAR WE, QU W, LI G, WAALKES MP. Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res*. 2003; 286:355–65. [PubMed: 12749863]
- TONG H, LIN M. Arsenic trioxide induced p15INK4B gene expression in myelodysplastic syndrome cell line MUTZ-1. *Zhonghua Xue Ye Xue Za Zhi*. 2002; 23:638–41. [PubMed: 12667347]
- TORANO EG, PETRUS S, FERNANDEZ AF, FRAGA MF. Global DNA hypomethylation in cancer: review of validated methods and clinical significance. *Clin Chem Lab Med*. 2012; 50:1733–42. [PubMed: 23089701]
- TSENG CH, HUANG YK, HUANG YL, CHUNG CJ, YANG MH, CHEN CJ, HSUEH YM. Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. *Toxicol Appl Pharmacol*. 2005; 206:299–308. [PubMed: 16039941]
- UDDIN AN, BURNS FJ, ROSSMAN TG, CHEN H, KLUZ T, COSTA M. Dietary chromium and nickel enhance UV-carcinogenesis in skin of hairless mice. *Toxicol Appl Pharmacol*. 2007; 221:329–38. [PubMed: 17499830]
- VAHTER M. Effects of arsenic on maternal and fetal health. *Annu Rev Nutr*. 2009; 29:381–99. [PubMed: 19575603]
- WAALKES MP, POIRIER LA. In vitro cadmium-DNA interactions: cooperativity of cadmium binding and competitive antagonism by calcium, magnesium, and zinc. *Toxicol Appl Pharmacol*. 1984; 75:539–46. [PubMed: 6474481]
- WADE PA. Methyl CpG-binding proteins and transcriptional repression. *Bioessays*. 2001; 23:1131–7. [PubMed: 11746232]
- WAISBERG M, JOSEPH P, HALE B, BEYERSMANN D. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*. 2003; 192:95–117. [PubMed: 14580780]
- WASSERMAN GA, LIU X, PARVEZ F, AHSAN H, FACTOR-LITVAK P, VAN GEEN A, SLAVKOVICH V, LOIACONO NJ, CHENG Z, HUSSAIN I, MOMOTAJ H, GRAZIANO JH. Water arsenic exposure and children's intellectual function in Arai-hazar, Bangladesh. *Environ Health Perspect*. 2004; 112:1329–33. [PubMed: 15345348]
- WEBER M, HELLMANN I, STADLER MB, RAMOS L, PAABO S, REBHAN M, SCHUBELER D. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet*. 2007; 39:457–66. [PubMed: 17334365]
- WEI M, WANIBUCHI H, MORIMURA K, IWAI S, YOSHIDA K, ENDO G, NAKAE D, FUKUSHIMA S. Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. *Carcinogenesis*. 2002; 23:1387–97. [PubMed: 12151359]
- WEISS A, KESHET I, RAZIN A, CEDAR H. DNA demethylation in vitro: involvement of RNA. *Cell*. 1996; 86:709–18. [PubMed: 8797818]

- WINSTON, H.; YU, CMH.; HARVEY, CHARLESF. Water Resources Research. 2003. Arsenic in groundwater in Bangladesh: A geostatistical and epidemiological framework for evaluating health effects and potential remedies; p. 39
- WU C, MORRIS JR. Genes, genetics, and epigenetics: a correspondence. Science. 2001; 293:1103–5. [PubMed: 11498582]
- WU F, BURNS FJ, ZHANG R, UDDIN AN, ROSSMAN TG. Arsenite-induced alterations of DNA photodamage repair and apoptosis after solar-simulation UVR in mouse keratinocytes in vitro. Environ Health Perspect. 2005; 113:983–6. [PubMed: 16079067]
- WU H, ZHANG Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. Genes Dev. 2011; 25:2436–52. [PubMed: 22156206]
- XIE Y, LIU J, BENBRAHIM-TALLAA L, WARD JM, LOGSDON D, DIWAN BA, WAALKES MP. Aberrant DNA methylation and gene expression in livers of newborn mice transplacentally exposed to a hepatocarcinogenic dose of inorganic arsenic. Toxicology. 2007; 236:7–15. [PubMed: 17451858]
- YAGI S, HIROSAWA M, SHIOTA K. DNA methylation profile: a composer-, conductor-, and player-orchestrated Mammalian genome consisting of genes and transposable genetic elements. J Reprod Dev. 2012; 58:265–73. [PubMed: 22790869]
- YAN YKT, ZHANG P, CHEN HB, COSTA M. Analysis of specific lysine histone H3 and H4 acetylation and methylation status in clones of cells with a gene silenced by nickel exposure. Toxicol Appl Pharmacol. 2003; 190:272–277. [PubMed: 12902198]
- YANG LQ, JI WD, TAO GH, ZHANG WJ, GONG CM, ZHOU L, LIU JJ, KE YB, ZHUANG ZX. Genome DNA hypomethylation in the process of crystalline nickel-induced cell malignant transformation. Zhonghua Yu Fang Yi Xue Za Zhi. 2010; 44:622–5. [PubMed: 21055078]
- YANG XD, HAN W, LIU F. DNA methylation in vertebrate embryogenesis. Yi Chuan. 2012; 34:1108–13. [PubMed: 23017451]
- YUAN TH, LIAN IEB, TSAI KY, CHANG TK, CHIANG CT, SU CC, HWANG YH. Possible association between nickel and chromium and oral cancer: a case-control study in central Taiwan. Sci Total Environ. 2011; 409:1046–52. [PubMed: 21195455]
- ZANG JJ, XIE F, XU JF, QIN YY, SHEN RX, YANG JM, HE J. P16 gene hypermethylation and hepatocellular carcinoma: a systematic review and meta-analysis. World J Gastroenterol. 2011; 17:3043–8. [PubMed: 21799651]
- ZHANG AH, BIN HH, PAN XL, XI XG. Analysis of p16 gene mutation, deletion and methylation in patients with arseniasis produced by indoor unventilated-stove coal usage in Guizhou, China. J Toxicol Environ Health A. 2007; 70:970–5. [PubMed: 17479413]
- ZHANG J, LI M, WU Y, FAN Y, ZHOU Y, TAN L, SHAO Z, SHI H. Methylation of RAR-beta2, RASSF1A, and CDKN2A genes induced by nickel subsulfide and nickel-carcinogenesis in rats. Biomed Environ Sci. 2011; 24:163–71. [PubMed: 21565688]
- ZHAO C, BU X. Promoter methylation of tumor-related genes in gastric carcinogenesis. Histol Histopathol. 2012; 27:1271–82. [PubMed: 22936446]

Figure 1. Methylation of a Normal Cell vs. Methylation of a Cancer Cell

Both hyper- and hypomethylation alterations occur in cancer. The figure lists the methylation differences of cancer cells vs. normal cells.

Figure 2.

Fig 2. Nickel-induced gene repression facilitated by chromatin condensation and DNA hypermethylation.

Nickel displaces magnesium in the DNA backbone and causes an initial state of chromatin condensation. Condensed chromatin recruits DNMT1 which hypermethylates the site. MeCP2 binds 5mC and recruits HDACs and corepressor complexes which will further promote chromatin condensation.

Table 1Human, Animal and *In Vitro* reports characterizing the carcinogenic effects of nickel compounds.

Study Type	Ref.	Exposure	Cell type or Animal model	Endpoint/Results
Human	(Qayyum et al., 2012)	inhalational/occupational	buccal cells	Increased micronuclei frequency in subjects occupationally exposed to nickel.
Human	(Sorahan and Williams, 2005)	inhalational/occupational	—	Increased risk of lung cancer in subjects occupationally exposed to nickel.
Human	(Arita et al., 2012)	inhalational/occupational	peripheral blood mononuclear cells (PBMCs)	PBMCs of subjects occupationally exposed to nickel displayed altered levels of histone modifications.
Human	(Yuan et al., 2011)	inhalational, oral	—	Subjects with oral cancer contained increased levels of nickel and chromium in the blood.
Animal bioassay	(Ottolenghi et al., 1975)	inhalational	F344 Rats	Rats exposed to nickel sulfide formed more lung tumors than control rats.
Animal bioassay	(Dunnick et al., 1995)	inhalational	F344/N Rats	Rats exposed to nickel oxide and nickel subsulfide formed an increased amount of lung neoplasms.
Animal bioassay	(Oller et al., 2008)	inhalational	Wistar rats CrI:WI (G1x/BRL/Han)	Rats exposed to metallic nickel displayed various lung alterations and dose-dependent increases in pheochromocytomas (males) and adenomas/carcinomas (females).
Animal bioassay	(Uddin et al., 2007)	oral	Skh1 nude mice	Mice exposed to nickel chloride and UVR contained more skin tumors than mice exposed to UVR alone.
<i>In vitro</i>	(Freitas et al., 2012)	chemical injected in medium	human neutrophil cell line	Ni (II) induced apoptosis of neutrophils and this event may be dependent on NADPH oxidase-derived reactive oxygen species.
<i>In vitro</i>	(Chen et al., 2010)	chemical injected in medium	human bronchial epithelial cells (BEAS-2b)	BEAS-2b cells treated with nickel chloride caused an increase of H3K9 dimethylation at the Sp1 promoter by inhibiting JMJD1A.
<i>In vitro</i>	(Biedermann KA, 1987)	chemical injected in media	human diploid fibroblasts	Nickel subsulfide induced anchorage independent growth without mutagenesis.
<i>In vitro</i>	(Miura et al., 1989)	chemical injected in media	mouse embryo cells	Nickel subsulfide induced morphological transformation without mutating the gene that confers ouabain resistance.

Table 2Human, Animal and *In Vitro* Reports Characterizing the Carcinogenic Effects of Arsenic Compounds.

Study Type	Ref.	Exposure	Cell type or Animal Model	Endpoint/Results
Human	(Meliker et al., 2007)	oral (drinking – water)	—	300–500 µg/l arsenic in drinking-water associated with bladder cancer.
Human	(Chen et al., 1985)	oral (drinking-water)	—	350–1140 µg/l arsenic in drinking-water associated with kidney and bladder cancer mortality.
Human	(Kurtio et al., 1999)	oral (drinking-water)	—	Low well water arsenic, 0.1–64 µg/l, associated with bladder cancer.
Human	(Leonardi et al., 2012)	oral (drinking-water)	—	Drinking-water arsenic (>100 µg/l) associated with basal cell carcinoma.
Human	(Winston et al., 2003)	oral (drinking-water)	—	21% of people in Bangladesh drinking from arsenic-contaminated wells had skin lesions.
Human	(Knobeloch et al., 2006)	oral (drinking-water)	—	Subjects who drank from arsenic-contaminated wells (>20 µg/l) for 10 years had an increased incidence of skin cancer.
Animal Bioassay	(Cui et al., 2006)	oral (drinking-water)	male A/J mice	Exposed mice (10 and 100 mg/l sodium arsenate) formed more tumors and larger tumors than control mice.
Animal Bioassay	(Wei et al., 2002)	oral (drinking-water)	F344 rats	Rats treated with 50 or 200 mg/l dimethylarsinic developed bladder cancers.
Animal Bioassay	(Shen et al., 2003)	oral (drinking-water)	F344 rats	Rats exposed to 200 mg/l of trimethylarsine oxide developed liver tumors.
<i>In Vitro</i>	(Pi et al., 2005)	chemical injected in media	human keratinocytes (HaCaT cells)	Cells treated with a low dose of sodium arsenite became apoptosis resistant.
<i>In Vitro</i>	(Chen et al., 2005)	chemical injected in media	mouse keratinocytes 291.03C	Cells treated with a short-term dose of arsenite (24h) reduced the amount of UVR-induced apoptosis.
<i>In Vitro</i>	(Biedermann et al., 1987)	chemical injected in media	human diploid fibroblasts	Sodium arsenate and sodium arsenite induced anchorage independent growth in cells.

Table 3Human, Animal, and *In Vitro* Investigations characterizing the carcinogenicity of cadmium compounds.

Study Type	Ref.	Exposure	Cell type or Animal Model	Endpoint/Results
Human	(Julin et al., 2012)	oral (dietary intake)	_____	Dietary cadmium exposure associated with prostate cancer incidence.
Human	(Satarug, 2012)	oral (dietary intake), inhalation (cigarette smoke)	_____	Cadmium exposure increases the risk of hepatocellular carcinoma by causing hepatogenous diabetes.
Human	(Adams et al., 2012)	oral, inhalation	_____	Urinary cadmium levels associated with cancer mortality.
Human	(Nawrot et al., 2006)	oral, inhalation	_____	Cadmium exposure was associated with lung cancer risk.
Animal Bioassay	(Waalkes and Rehm, 1992)	oral	Wistar rats (WF/NCr)	Exposure to 100 and 200 mg/l cadmium chloride increased the incidence of leukemia, prostate tumors, and testis tumors in rats.
Animal Bioassay	(Glaser U, 1990)	inhalation	Wistar rats (TNO/W75)	Rats exposed via inhalation to 0–900 µg/m ³ of various cadmium species displayed an increase in lung tumor incidence.
Animal Bioassay	(Takenaka et al., 1983)	inhalation	Wistar rats (TNO/W75)	Rats exposed to 25 or 50 µg/m ³ of cadmium chloride via inhalation developed lung carcinomas.
<i>In Vitro</i>	(Son et al., 2012)	chemical injected in media	human bronchial epithelial cells (BEAS-2b)	0.5–2 µM cadmium increased the transformative, migratory, and invasive properties of cells and activated PI3K, AKT, GSK-3β, and β-catenin signaling pathways. These events were mediated by ROS.

Table 4

Length of Exposure of Cadmium and Epigenetic Outcome

Reference	Length of Exposure	Methylation Event	DNMT Levels
Benbrahim-Tallaa et al., 2007	Long-term (10 wks)	Global Hypermethylation & Gene-specific Hypermethylation	Overexpression of DNMT3b and Increased DNMT Activity
Takiguchi et al., 2003	Long-term (10wks)	Global Hypermethylation	Increased DNMT Activity
Jing et al., 2008	Long-term (2 months)	Global Hypermethylation	Overexpression of DNMT1, DNMT3a/b and Increased DNMT Activity
Inglot et al., 2012	Short-term (72 h)	Global Hypermethylation	N/A
Hossain et al., 2012	Long-term (Lifelong)	Global Hypomethylation	Low expression of DNMT3b
Huang et al., 2008	Short-term (24–48 h)	Global Hypomethylation	N/A
Takiguchi et al., 2003	Short-term (1 wk)	Global Hypomethylation	Decreased DNMT Activity

Table 5

Human, animal bioassay and in vitro investigations characterizing the carcinogenicity of Cr (VI).

Study Type	Ref.	Exposure	Cell Type or Animal Model	Results
Human	(Koh et al., 2011)	inhalation	—	Workers exposed to Cr (VI)-containing cement dust had increased incidence of stomach cancer.
Human	(Halasova et al., 2009)	inhalation	—	Cr (VI) exposed workers developed lung cancer at an early age and have a higher incidence of small cell lung carcinoma than unexposed lung cancer patients.
Human	(Birk et al., 2006)	inhalation	—	Cr (VI) exposed workers with chromium urine levels of > 200 µg/l had an increased lung cancer risk.
Human	(Linos et al., 2011)	oral (drinking-water)	—	Subjects orally exposed to Cr (VI) displayed elevated cancer mortality. The exposed population experienced high standard mortality rates for liver, lung, and kidney cancers.
Animal Bioassay	(Stout et al., 2009)	oral (drinking-water)	F344/N rats and B6C3F1 mice	Rats exposed to 516 mg/l SDD for 2 years developed oral neoplasms. Mice exposed to 257 mg/l SDD for 2 years developed small intestinal neoplasms.
Animal Bioassay	(Davidson et al., 2004)	oral (drinking-water)	CRL: SK1-hrBR hairless mice	Mice co-exposed to potassium chromate (2.5 and 5 mg/l) and UV formed more skin tumors than mice exposed to either potassium chromate alone or UV alone.
In Vitro	(Sun et al., 2011)	chemical injected in media	human bronchial epithelial cells (BEAS-2b)	Cr (VI) transformed cells displayed altered gene expressions of cancer-related genes such as desmocollins, cyclin D1, and TGFβ2.
In Vitro	(Kim A. Biedermann, 1987)	chemical injected in media	human fibroblasts	Cr (VI) compounds induce a mutation that confers resistance to 6-thioguanine and causes anchorage independent growth.