Published in final edited form as:

J Trace Elem Med Biol. 2015 July ; 31: 209–213. doi:10.1016/j.jtemb.2014.04.001.

10th NTES Conference: Nickel and arsenic compounds alter the epigenome of peripheral blood mononuclear cells

Jason Brocato and Max Costa*

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

Abstract

The mechanisms that underlie metal carcinogenesis are the subject of intense investigation ; however, data from *in vitro* and *in vivo* studies are starting to piece together a story that implicates epigenetics as a key player. Data from our lab has shown that nickel compounds inhibit dioxygenase enzymes by displacing iron in the active site. Arsenic is hypothesized to inhibit these enzymes by diminishing ascorbate levels- an important co-factor for dioxygenases. Inhibition of histone demethylase dioxygenases can increase histone methylation levels, which also may affect gene expression. Recently, our lab conducted a series of investigations in human subjects exposed to high levels of nickel or arsenic compounds. Global levels of histone modifications in peripheral blood mononuclear cells (PBMCs) from exposed subjects were compared to low environmentally exposed controls. Results showed that nickel increased H3K4me3 and decreased H3K9me2 globally. Arsenic increased H3K9me2 and decreased H3K9ac globally. Other histone modifications affected by arsenic were sex-dependent. Nickel affected the expression of 2,756 genes in human PBMCs and many of the genes were involved in immune and carcinogenic pathways. This review will describe data from our lab that demonstrates for the first time that nickel and arsenic compounds affect global levels of histone modifications and gene expression in exposed human populations.

Keywords

Arsenic; Nickel; histones modifications; epigenetics; gene expression; PBMCs; metals; human exposure

Introduction

Carcinogenic metals are ubiquitous elements and humans are exposed to these toxicants via air, drinking water, occupational settings, and consumer products. Altering the epigenome is

© 2014 Elsevier GmbH. All rights reserved.

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

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement

None of the authors have a conflict of interest.

one of the underlying mechanisms that characterize the carcinogenicity of metallic compounds [1]. Arsenic and nickel are two carcinogenic metals that have been documented by many investigations to alter the epigenetic landscape of a cell to mirror the epigenome of a cancer cell [2]. A great number of epidemiological studies have established the carcinogenicity of arsenic and nickel compounds by associating metal exposure with human cancer incidence [3-6].

Histone modifications are an epigenetic mark that can be affected by metal exposure [1, 7]. Histone proteins may be modified post-translation with various functional groups including-methylation, acetylation, phosphorylation, sumoylation, and ubiquitination. The amino acids that comprise the histone tail are very susceptible to post-translational modifications, such as lysine 9 of histone 3 (H3K9). This amino acid can receive mono- (H3K9me), di- (H3K9me2), or tri- (H3K9me3) methylation marks or acetylation (H3K9ac). Histone modifications can be associated with gene activation (H3K9ac) or gene repression (H3K9me2) [7, 8]. Nickel and arsenic compounds have been found to interfere with the epigenetic machinery that erases histone methylation marks [9]. Thus, exposure to these metals affects the levels of histone methylation in the cell and this can influence gene expression. For a detailed review of histone modifications and their function, the reader is referred to Choudhuri et al. 2010 [8].

Human populations exposed to large levels of carcinogenic metals offer a unique opportunity to study the mechanisms that underlie metal carcinogenesis. Recently, our lab conducted a series of investigations on human subjects exposed to high levels of nickel or arsenic compounds. Previous data from our lab demonstrated that these metals affect the methylation levels of H3K4 and H3K9 possibly due to inhibition of dioxygenases-demethylases that remove methylation marks from histones and DNA. To investigate if this phenomenon occurs *in vivo*, these marks were examined in PBMCs from highly exposed subjects and were compared to low environmentally exposed controls. This article reviews data from our lab that shows for the first time that nickel and arsenic compounds affect global levels of histone modifications in humans.

Nickel

Background

Nickel compounds, inclusive of water-soluble salts, are known human carcinogens and occupational exposure to these compounds is a concern [1]. Volcanic eruptions, meteors, soils, ocean floors, and ocean water are all natural sources of nickel [10, 11]. Manmade sources of nickel can be found in many products such as coins, jewelry, stainless steel, batteries, and medical devices [2]. Compounds containing nickel have been found to cause oxidative stress [12], hematotoxicity [13], and immunotoxicity [14]. The largest concern for human exposure to nickel is from occupational exposure. Occupational exposure to nickel compounds occurs during nickel refinery, plating, and welding operations [15]. Many studies have demonstrated nickel's ability to induce cancer; however, its mutagenic capabilities are low [10]. Given nickel's large effect on the epigenetic state of cells, it is likely that nickel- induced carcinogenesis occurs via epigenetic mechanisms. Nickel compounds are not very toxic to the cell [16], which allows for the survival of cells

epigenetically disturbed by nickel and this “damaged yet survivable” state may initiate carcinogenesis.

Uptake of nickel into the cell is dependent on the particle's size, charge, surface characteristics, and structure. Insoluble nickel compounds such as nickel sulfides and oxides are considered much more potent carcinogens than soluble nickel compounds [17]; however, the charge of the compound also plays an important role in their uptake [18]. Crystalline nickel subsulfide, which is a poorly water soluble nickel compound, can enter the cell via phagocytosis/endocytosis and is one of the most tumorigenic forms of all nickel compounds; whereas, amorphous nickel sulfide, another sparingly soluble nickel compound, does not enter cells and has low cellular toxicity [18].

Mechanisms underlying epigenetic changes induced by nickel compounds

Previously, our lab has illustrated some of the roles of nickel compounds after they enter the cell. Once in the cell, nickel can affect histone modifications by inhibiting dioxygenases [19] or histone acetyltransferases (HATs). Nickel's potential to induce gene silencing is mediated by increased condensation of the genomic region followed by DNA methylation both of which facilitate heterochromatinization [20]. Nickel compounds may affect DNA methylation levels via its influence on the activity of ten-eleven translocation (TET) proteins enzyme activity [2].

1. Nickel inhibits histone modifying enzymes—The major targets of nickel ions are the dioxygenase family of enzymes and they require iron, ascorbate, oxygen, and α -ketoglutarate as co-factors. Nickel has been shown to displace iron in the active site of these enzymes, which inhibits their enzymatic function [19]. A study by Chen et al. 2010 found that nickel binds to the iron binding motif in dioxygenases with three times more affinity than iron [21]. Nickel's allosteric inhibition of dioxygenases, inactivates the dioxygenase in an irreversible manner. Dioxygenases have a large influence on the epigenetic landscape of the cell and inhibition can lead to an increase in histone methylation marks because many histone demethylases are dioxygenases [22, 23]. JMJD1A demethylates H3K9me2 and our lab has previously shown that nickel exposure increases global levels of H3K9me2 via inhibition of JMJD1A in human bronchial epithelial cells (BEAS-2b)[22]. Chromatin immunoprecipitation revealed an increase in H3K9me2 at promoters of down-regulated genes [22, 23], which illustrates nickel's potential to affect gene expression. The *SPRY2* promoter was found to be a downstream target of the H3K9me2 JMJD1A demethylase and chronic treatment of BEAS-2b cells to nickel silenced *SPRY2* expression, which promoted nickel-induced anchorage-independent growth [22]. Also, we have reported that H3K4me3 levels are increased and H3K9me2 levels are decreased after nickel exposure in human lung carcinoma A549 cells [9], and these effects are likely due to demethylase inhibition.

Nickel compounds may also affect histone modifications via other mechanisms besides inhibition of dioxygenases. Acetylated lysines, which are a target mark of proteins with bromo domains and associated with gene activation, were found to be reduced globally by nickel and nickel compounds in *in vitro*, animal, and human studies [24-26]. Ni^{+2} binds to histidine 18 on the histones' N-terminal end and prevents acetylation [24, 27]. A study by

Kang et al. 2003 found that nickel chloride decreases histone 4 acetylation levels by inhibiting histone acetyltransferase activity in a dose-dependent manner [28].

2. Nickel induces heterochromatinization—Many of nickel's effects on histone modifications are associated with gene repression- increased H3K9me2 and global hypoacetylation. Nickel-induced heterochromatinization further ensures the silencing of genes by their incorporation into heterochromatic regions. Nickel- induced heterochromatinization was first described in the G10 and G12 cell lines. 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 but not in the G10 line where the *gpt* gene was placed in a region of euchromatin [29]. G12 cells treated with nickel were found to be resistant to DNase I digestion and nickel-treated G10 cells were not. The resistance was significantly less in cells treated with magnesium and non-existent in cells treated with cobalt [30].

Later, it was shown that nickel-induced heterochromatinization was caused by nickel displacing magnesium (Mg) in heterochromatic complexes. Mg^{2+} 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 Mg^{2+} resulting in the condensation of euchromatin into heterochromatin [20].

3. Nickel inhibits erasers of DNA methylation—In addition to inducing its incorporation into heterochromatin, nickel silences the *gpt* gene by inducing hypermethylation at the *gpt* loci. 5-aza-cytidine, a potent inhibitor of DNA methyltransferases, was shown to reactivate the *gpt* gene in G12 cells treated with nickel sulfide [30]. 5-methylcytosine sensitive restriction enzymes confirmed hypermethylation of the *gpt* gene [30] and the 5-methylcytosine binding protein MeCP2 was increased at the *gpt* gene in nickel-silenced clones but not in parental G12 cells [20].

A number of studies have demonstrated nickel's ability to induce promoter hypermethylation and subsequent silencing of tumor suppressor genes *in vivo*. A study by Govindarajan et al. 2002 found that mice implanted with nickel sulfide developed tumors with the *P16* gene hypermethylated [31]. Wistar rats given an intramuscular injection of 10 mg nickel subsulfide developed muscle tumors that showed 5' hypermethylation of *RARβ2*, *RASSF1A*, and *P16* [32]. The O^6 -methylguanine DNA methyltransferase (*MGMT*) gene, which encodes an enzyme that repairs O^6 -methylguanine, was hypermethylated in nickel sulfide-transformed human bronchial epithelial (16HBE) cells [33].

All of the above instances of nickel-induced hypermethylation may be described by nickel's ability to inhibit ten-eleven translocation (TET) proteins. TET proteins are dioxygenases that mediate DNA demethylation. TET dioxygenases convert 5-methylcytosine to 5-hydroxymethylcytosine and deaminases in the cell deaminate 5-hydroxymethylcytosine to 5-hydroxyuracil. The U:G mismatch is recognized by DNA repair enzymes of the base excision repair (BER) pathway and the U is replaced with unmethylated cytosines. Alternative mechanisms of TET-mediated DNA demethylation involve formation of the intermediates 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). Thymine DNA

glycosylase of the BER pathway recognizes 5fC and 5-caC and replaces them with unmethylated cytosine. Also, decarboxylases can decarboxylate 5-caC to generate unmethylated cytosine [2].

Occupational exposure to nickel compounds alters global levels of histone modifications and gene expression in PBMCs

In order to determine if the changes in epigenetic marks observed with *in vitro* exposure to nickel compounds also occurred in humans who were occupationally exposed to nickel, our lab established a collaboration with Lanzhou university in China to collect biological samples including blood, urine, and plasma from nickel-refinery workers who were occupationally exposed to high levels of nickel oxide (NiO) from inhalation of nickel dusts. 45 study participants who were classified as healthy males between 24 and 56 years old who worked in the nickel-refinery in Jinchang, Gansu, China for at least 3 years were selected. 75 age-matched controls living in Gansu, China with no previous nickel exposure except through environmental sources were also selected. The nickel-refinery in Jinchang experiences ambient air nickel concentrations of 1 mg/m³, compared to US ambient air levels of 5-50 ng/m³. The population in Jinchang experiences a 4-fold higher incidence of lung cancer than the general population [34].

To investigate global levels of histone modifications affected by occupational exposure to nickel, our lab conducted ELISAs investigating the histone marks-H3K4me3, H3K9me2, and H3K9ac- in peripheral blood mononuclear cells (PBMCs) and results were published in Arita et al. 2012 [34]. Occupationally exposed subjects displayed an increase in H3K4me3 and a decrease in H3K9me2 compared to environmentally exposed subjects. H3K9ac was not significantly changed between populations however it was negatively correlated with urinary nickel levels. Also, H3K4me3 levels were positively correlated with urinary nickel and this phenomenon was likely due to nickel's inhibition of the H3K4me3 demethylase, JARID1A [34]. Our lab previously demonstrated that H3K4me3 increased in hypoxia due to inhibition of JARID1A [35] and given the hypoxia-like effects seen after nickel exposure, it is likely that increased H3K4me3 is due to JARID1A inhibition. The observance of decreased H3K9ac with higher urinary nickel supports previous findings from our lab that nickel inhibits histone acetyltransferases to lower acetylated histone levels *in vitro* [28]. Also, the nickel-induced increase of global H3K4me3 correlates with previous *in vitro* investigations from our lab [9]. The decrease in H3K9me2 was unexpected and does not agree with our previous *in vitro* data [23]; however, it is not surprising for a metal to exhibit opposite effects in different cell types. Interindividual variances of H3K4me3, H3K9ac, and H3K9me2 were larger compared with intraindividual variance in both populations indicating that these modifications are true epigenetic marks due to their sustainability [34].

Affymetrix exon arrays were used to analyze the mRNA profiles of PBMCs from 8 nickel-refinery workers and 10 referents and results were published in Arita et al. 2013 [36]. A total of 31 genes were changed more than 2-fold in all subjects with occupational exposure to high levels of nickel compared to subjects with environmental exposure to lower levels of nickel, including 16 down-regulated and 15 up-regulated genes. There was a total of 2,756 differentially expressed genes between the workers and controls with almost 2,000 of those

genes being down-regulated. The largest group of differentially expressed genes was cytokines and chemokines, such as *CCL20*, *CCR2*, *CX3CR1*, *IL1A*, *IL6*, and *IL1RN*, involved in chemotaxis and cytokine-to-cytokine signaling pathways. Other gene families represented in the list of differentially expressed genes included transcription factors, protein kinases, oncogenes, tumor suppressors, and cell differentiation markers [36].

The genes changed *in vivo* in the nickel-refinery workers were compared to differentially expressed genes in nickel-exposed PBMCs *in vitro*. When genes at the 1.5-fold change level were compared between studies, it was observed that only 9 genes were the same- CD14, FUCA1, DAPK1, HMOX1, FGL2, IGF6, FCGR1B, TLR8, and CLEC7A. However, when data from the nickel-refinery workers were compared with another *in vivo* study [37] that investigated mRNA profiles of subjects with nasopharyngeal cancers, there was an overwhelming overlap of differentially expressed genes [36]. Nickel is an established nasal carcinogen and occupational exposure to nickel compounds has been associated with nasal cancer incidence [38]. These comparisons highlight the importance of *in vivo* studies in establishing biomarkers for disease and suggest that *in vivo* human studies surpass *in vitro* studies that control for cell type when observing the effects of nickel compounds.

Arsenic

Background

Arsenic exposure has been associated with skin, lung, liver and bladder cancers [39] as well as noncarcinogenic outcomes, including cardiovascular disease and neurological deficits [40, 41]. Arsenic displays both acute and chronic toxicity and has no known biological function. Arsenic has been demonstrated to be a causative agent in the development of skin, lung, and bladder cancers [42, 43]. 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 [44]. Various epidemiological studies have linked Arsenic with cancer incidence in humans [3, 4, 45].

Mechanisms underlying epigenomic changes induced by arsenic compounds

Arsenic has been demonstrated to create oxidative stress in cells and creation of oxidative free radicals may affect the activity of histone demethylases in the cell. Arsenic works to accumulate reactive oxygen species within the cell by vacillating between its oxidation states, promoting the inflammatory response, interfering with antioxidant enzymes, and increasing the amount of free iron in the cell [46]. Arsenic is largely associated with cancer incidence but its ability to create mutations is poor despite its capacity to create large amounts of oxidative stress [2]. However, arsenic-induced oxidative stress is linked to the main genre of mechanisms that underlie arsenic-induced carcinogenesis- epigenetics.

One co-factor pertinent to the function of dioxygenases is ascorbate. Arsenic-induced oxidative stress will deplete cellular levels of ascorbate and inhibit the oxidative histone demethylases [46] and has potential to inhibit TET proteins, which mediate DNA demethylation. Organic arsenic species can increase levels of bioavailable iron by stimulating its release from ferritin, an iron-binding protein in the cell. Iron mediates the generation of oxygen radicals such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2),

and the very potent hydroxyl radical ($\bullet\text{OH}$) via Fenton reactions and Haber-Weiss reactions [46].

The assertion that arsenic affects histone methylation due to depletion of ascorbate levels and subsequent inhibition of dioxygenases is a plausible hypothesis; however, data supporting this theory is mixed. Human urothelial cells transformed by arsenic displayed decreased levels of H3K27me3 and H3K9me2 and increased levels of H3K4me2 in the promoter region of the *WNT5A* gene [47]. Human lung carcinoma A549 cells treated with inorganic arsenite displayed global increases in H3K9me2 and H3K4me3 but a decrease in H3K27me3 was observed. The increase in H3K9me2 was accompanied by an increase in G9a protein levels, the histone methyltransferase that methylates H3K9me1 [48].

The established association between arsenic and both global and gene-specific hypermethylation [2] may also be linked to arsenic-induced oxidative stress given that TET proteins are dioxygenases that also require ascorbate as a co-factor; however, this hypothesis is weakened from the same issue of conflicting data to support its theory. Arsenic is associated with both hyper- and hypomethylation occurrences on both the global and gene-specific levels [2].

Arsenic-induced changes in global histone modifications in PBMCs from human subjects

Previous *in vitro* data from our lab demonstrated that arsenic compounds increase global levels of H3K4me3 and H3Kme2 in human lung carcinoma A549 cells [9]. In order to observe the expected tendency of arsenic to affect global histone modifications *in vivo*, PBMC's from subjects exposed to high levels of arsenic in drinking- water were examined and results were published in Chervona et al. 2012 [49]. A subset of 40 subjects from a folate clinical trial in Bangladesh (FACT study) comprised of 20 subjects in a high exposure group (165.0 $\mu\text{g/L}$ urinary arsenic) and 20 subjects in a low exposure group (69.0 $\mu\text{g/L}$ urinary arsenic) with equal amounts of males and females were examined. The current maximum arsenic levels in US drinking-water is 10 $\mu\text{g/L}$ compared to 50-500 $\mu\text{g/L}$ at the Bangladesh study sites [49].

Results from the investigation showed that global H3K9me2 levels correlated positively with urinary arsenic, while global H3K9ac levels correlated negatively [49]. Both of these incidences are associated with gene repression and coincides with the postulation that H3K9 acetylation must decrease in order to make room for more H3K9 methylation. The increase in H3K9me2 concurs with our *in vitro* data [9] and alludes to the inhibition of JMJD1A demethylases. The arsenic-induced oxidative stress is likely to have reduced cellular levels of ascorbate thereby inhibiting the dioxygenase enzymes. Observed changes in other histone modifications were found to be sex-dependent. The acetylation marks H3K18ac and H3K27ac were higher in males, while methylation marks H3K4me3 and H3K27me3 were higher in females. Chervona et al. hypothesized that this observance may be due to the effects of estrogen on an enhancer for *EZH2* gene, the histone methyltransferase that methylates H3K27 [49, 50]. Investigations examining the differences between male and female gene expression profiles of subjects from the FACT study are currently underway

and may shed light on possible mechanisms that would elucidate the influence of sex on global changes of histone modifications induced by arsenic compounds.

Conclusion

The mechanisms that underlie metal carcinogenesis are still vague; however, data from *in vitro* and *in vivo* studies are starting to piece together a story that implicates epigenetics as a key player. Data from our lab has shown that nickel inhibits dioxygenase enzymes by displacing iron in the active site. Arsenic is hypothesized to inhibit these enzymes by diminishing ascorbate levels- another important co-factor for dioxygenases. Inhibition of dioxygenases can increase histone methylation and this phenomenon is exhibited in Zhou et al. 2009 [9]- *in vitro* exposure to nickel or arsenic compounds increased H3K4me3 and H3K9me2, Arita et al. 2012 [34]- occupational exposure to nickel compounds increased H3K4me3 in human PBMCs, and Chervona et al. 2012 [49]- high environmental exposure to arsenic compounds increased H3K9me2 in human PBMCs. Nickel altered the expression of 2,756 genes in PBMCs from humans occupationally exposed and many of the genes were involved in immune and carcinogenic pathways. Further studies are required to establish these findings as epigenetic signatures of metal exposure. When dealing with human populations, researchers must consider total exposure profiles of individuals as many toxicants or combinations of toxicants may contribute to the endpoints. Animal studies have the potential to validate findings of human studies because they offer complete experimental control and it would be interesting to see if these results could be replicated in mice exposed to nickel via whole body inhalation or arsenic via drinking-water.

Acknowledgments

Funding

This work was supported by the National Institute of Environmental Health Sciences (NIEHS) by grants [R01ES023174, P30ES000260, P42ES010349 and P30CA016087].

References

1. Arita A, Costa M. Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium. *Metallomics*. 2009; 1(3):222–8. [PubMed: 20461219]
2. Brocato J, Costa M. Basic mechanics of DNA methylation and the unique landscape of the DNA methylome in metal-induced carcinogenesis. *Crit Rev Toxicol*. 2013; 43(6):493–514. [PubMed: 23844698]
3. Kurtio P, et al. Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. *Environ Health Perspect*. 1999; 107(9):705–10. [PubMed: 10464069]
4. Meliker JR, N.J. Arsenic in drinking water and bladder cancer: review of epidemiological evidence. *Trace Metals and other Contaminants in the Environment*. 2007; (9):551–584.
5. Sorahan T, Williams SP. Mortality of workers at a nickel carbonyl refinery, 1958-2000. *Occup Environ Med*. 2005; 62(2):80–5. [PubMed: 15657188]
6. Yuan TH, et al. Possible association between nickel and chromium and oral cancer: a case-control study in central Taiwan. *Sci Total Environ*. 2011; 409(6):1046–52. [PubMed: 21195455]
7. Chervona Y, Arita A, Costa M. Carcinogenic metals and the epigenome: understanding the effect of nickel, arsenic, and chromium. *Metallomics*. 2012; 4(7):619–27. [PubMed: 22473328]
8. Choudhuri S,CY, Klaassen CD. Molecular targets of epigenetic regulation and effectors of environmental influences. *Toxicol Appl Pharmacol*. 2010; 245(3):378–393. [PubMed: 20381512]

9. Zhou X, et al. Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. *Toxicol Appl Pharmacol.* 2009; 236(1):78–84. [PubMed: 19371620]
10. 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(1):63–72. [PubMed: 7683771]
11. Cruz MJ, et al. [Occupational asthma caused by chromium and nickel]. *Arch Bronconeumol.* 2006; 42(6):302–6. [PubMed: 16827980]
12. Schmid M, et al. 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(3):385–90. [PubMed: 17250893]
13. Salnikow K, et al. The involvement of hypoxia-inducible transcription factor-1-dependent pathway in nickel carcinogenesis. *Cancer Res.* 2003; 63(13):3524–30. [PubMed: 12839937]
14. Hostynek JJ. Sensitization to nickel: etiology, epidemiology, immune reactions, prevention, and therapy. *Rev Environ Health.* 2006; 21(4):253–80. [PubMed: 17243350]
15. Salnikow K, Zhitkovich A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem Res Toxicol.* 2008; 21(1):28–44. [PubMed: 17970581]
16. Costa M, et al. Nickel carcinogenesis: epigenetics and hypoxia signaling. *Mutat Res.* 2005; 592(1-2):79–88. [PubMed: 16009382]
17. Ouyang W, et al. Soluble and insoluble nickel compounds exert a differential inhibitory effect on cell growth through IKK α -dependent cyclin D1 down-regulation. *J Cell Physiol.* 2009; 218(1):205–14. [PubMed: 18792914]
18. Munoz A, Costa M. Elucidating the mechanisms of nickel compound uptake: a review of particulate and nano-nickel endocytosis and toxicity. *Toxicol Appl Pharmacol.* 2012; 260(1):1–16. [PubMed: 22206756]
19. Giri NC, P.L. Sun H, Zoroddu MA, Costa M, Maroney MJ. Structural investigations of the nickel-induced inhibition of truncated constructs of the JMJD2 family of histone demethylases using X-ray absorption spectroscopy. *Biochemistry.* 2013; 52(24):4168–4183. [PubMed: 23692052]
20. Ellen TP, et al. Heterochromatinization as a potential mechanism of nickel-induced carcinogenesis. *Biochemistry.* 2009; 48(21):4626–32. [PubMed: 19338343]
21. Chen H, et al. Nickel ions inhibit histone demethylase JMJD1A and DNA repair enzyme ABH2 by replacing the ferrous iron in the catalytic centers. *J Biol Chem.* 2010; 285(10):7374–83. [PubMed: 20042601]
22. Chen H, et al. Hypoxia and nickel inhibit histone demethylase JMJD1A and repress Spry2 expression in human bronchial epithelial BEAS-2B cells. *Carcinogenesis.* 2010; 31(12):2136–44. [PubMed: 20881000]
23. Chen H, et al. Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. *Mol Cell Biol.* 2006; 26(10):3728–37. [PubMed: 16648469]
24. Broday L, et al. Nickel compounds are novel inhibitors of histone H4 acetylation. *Cancer Res.* 2000; 60(2):238–41. [PubMed: 10667566]
25. Golebiowski F, Kasprzak KS. Inhibition of core histones acetylation by carcinogenic nickel(II). *Mol Cell Biochem.* 2005; 279(1-2):133–9. [PubMed: 16283522]
26. Ke Q, et al. Alterations of histone modifications and transgene silencing by nickel chloride. *Carcinogenesis.* 2006; 27(7):1481–8. [PubMed: 16522665]
27. Zoroddu MA, et al. Interaction of Ni(II) and Cu(II) with a metal binding sequence of histone H4: AKRHRK, a model of the H4 tail. *Biochim Biophys Acta.* 2000; 1475(2):163–8. [PubMed: 10832031]
28. Kang J, et al. Nickel-induced histone hypoacetylation: the role of reactive oxygen species. *Toxicol Sci.* 2003; 74(2):279–86. [PubMed: 12773760]
29. Klein CB, Costa M. DNA methylation, heterochromatin and epigenetic carcinogens. *Mutat Res.* 1997; 386(2):163–80. [PubMed: 9113117]
30. Lee YW, et al. Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol Cell Biol.* 1995; 15(5):2547–57. [PubMed: 7537850]

31. Govindarajan B, et al. Reactive oxygen-induced carcinogenesis causes hypermethylation of p16 (INK4a) and activation of MAP kinase. *Mol Med.* 2002; 8(1):1–8. [PubMed: 11984000]
32. Zhang J, et al. Methylation of RAR-beta2, RASSF1A, and CDKN2A genes induced by nickel subsulfide and nickel-carcinogenesis in rats. *Biomed Environ Sci.* 2011; 24(2):163–71. [PubMed: 21565688]
33. Ji W, et al. Epigenetic silencing of O6-methylguanine DNA methyltransferase gene in NiS-transformed cells. *Carcinogenesis.* 2008; 29(6):1267–75. [PubMed: 18204074]
34. Arita A, et al. Global levels of histone modifications in peripheral blood mononuclear cells of subjects with exposure to nickel. *Environ Health Perspect.* 2012; 120(2):198–203. [PubMed: 22024396]
35. Zhou X, et al. Hypoxia induces trimethylated H3 lysine 4 by inhibition of JARID1A demethylase. *Cancer Res.* 2010; 70(10):4214–21. [PubMed: 20406991]
36. Arita A, MA, Chervona Y, Niu J, Qu Q, Zhao N, Ruan Y, Kiok K, Kluz T, Sun H, Clancy HA, Shamy M, Costa M. Gene expression profiles in peripheral blood mononuclear cells of Chinese nickel refinery workers with high exposures to nickel and control subjects. *Cancer Epidemiol Biomarkers Prev.* 2013; 22(2):261–269. [PubMed: 23195993]
37. Dodd LE, et al. Genes involved in DNA repair and nitrosamine metabolism and those located on chromosome 14q32 are dysregulated in nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2006; 15(11):2216–25. [PubMed: 17119049]
38. Torjussen W. Occupational nasal cancer caused by nickel and nickel compounds. *Rhinology.* 1985; 23(2):101–5. [PubMed: 4035236]
39. Jensen TJ, et al. Epigenetic remodeling during arsenical-induced malignant transformation. *Carcinogenesis.* 2008; 29(8):1500–8. [PubMed: 18448484]
40. Tseng CH, et al. Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. *Toxicol Appl Pharmacol.* 2005; 206(3):299–308. [PubMed: 16039941]
41. Wasserman GA, et al. Water arsenic exposure and children's intellectual function in Araihaazar, Bangladesh. *Environ Health Perspect.* 2004; 112(13):1329–33. [PubMed: 15345348]
42. Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr Eval Carcinog Risks Hum.* 2004; 84:1–477. [PubMed: 15645577]
43. Schuhmacher-Wolz U, et al. Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects. *Crit Rev Toxicol.* 2009; 39(4):271–98. [PubMed: 19235533]
44. Kinniburgh DG, Kosmus W. Arsenic contamination in groundwater: some analytical considerations. *Talanta.* 2002; 58(1):165–80. [PubMed: 18968743]
45. Leonardi G, et al. Inorganic arsenic and basal cell carcinoma in areas of Hungary, Romania, and Slovakia: a case-control study. *Environ Health Perspect.* 2012; 120(5):721–6. [PubMed: 22436128]
46. Chervona Y, Costa M. The control of histone methylation and gene expression by oxidative stress, hypoxia, and metals. *Free Radic Biol Med.* 2012; 53(5):1041–7. [PubMed: 22841757]
47. Jensen TJ, et al. Epigenetic mediated transcriptional activation of WNT5A participates in arsenical-associated malignant transformation. *Toxicol Appl Pharmacol.* 2009; 235(1):39–46. [PubMed: 19061910]
48. Zhou X, et al. Arsenite alters global histone H3 methylation. *Carcinogenesis.* 2008; 29(9):1831–6. [PubMed: 18321869]
49. Chervona Y, et al. Associations between arsenic exposure and global posttranslational histone modifications among adults in Bangladesh. *Cancer Epidemiol Biomarkers Prev.* 2012; 21(12):2252–60. [PubMed: 23064002]
50. Bredfeldt TG, et al. Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. *Mol Endocrinol.* 2010; 24(5):993–1006. [PubMed: 20351197]