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

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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 2756 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.

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 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. [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.

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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].

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].

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].

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