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THE EFFECT OF EXPOSURE TO CARCINOGENIC METALS ON HISTONE TAIL MODIFICATIONS AND GENE EXPRESSION IN HUMAN SUBJECTS

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Abstract

The precise mechanisms for the carcinogenesis of nickel and arsenic compounds are not completely understood. In recent years, alterations of epigenetic mechanisms have been implicated in the carcinogenesis of these two metal compounds. *In vitro* exposure to nickel or arsenic induces changes in both DNA methylation patterns, as well as, in the levels of posttranslational modifications of histone tails. Changes in DNA methylation patterns have been reported in human subjects exposed to arsenic. Here we review our recent reports on the alterations in global levels of posttranslational histone modifications in peripheral blood mononuclear cells (PBMCs) of subjects with occupational exposure to nickel and subjects exposed to arsenic in their drinking water. Occupational exposure to nickel was associated with an increase in H3K4me3 and decrease in H3K9me2. A global increase in H3K9me2 and decrease in H3K9ac was found in subjects exposed to arsenic. Additionally, exposure to arsenic resulted in opposite changes in a number of histone modifications in males compared to females. The results of these two studies suggest that exposure to nickel or arsenic compounds, and possibly other carcinogenic metal compounds, can induce changes in global levels of posttranslational histone modifications in peripheral blood mononuclear cells.

Keywords

nickel; arsenic; epigenetics; histone tail modifications

Introduction

Epidemiological, cell culture, and animal experimental studies have shown an increased cancer incidence associated with chronic exposure to various metals including nickel and arsenic [1–3]. For example, occupational exposure to nickel compounds has been associated

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with lung and nasal cancers, while exposure to arsenic has been associated with skin, lung, bladder, kidney, and liver cancers [4, 5]. The carcinogenic effects of metal compounds have been linked to alterations in signaling pathways, DNA damage via both oxidative and non-oxidative (DNA adducts) mechanisms, and activation or silencing of gene expression through epigenetic mechanisms including changes in DNA methylation patterns and changes in levels of posttranslational histone modifications [1].

The precise mechanisms by which nickel and arsenic compounds exert their carcinogenic properties are not completely understood. In the last decade, alterations of epigenetic mechanisms have been implicated in the actions of both nickel and arsenic's carcinogenic properties. Studies have shown that *in vitro* exposure to nickel compounds results in an intracellular accumulation of nickel ions, changes in DNA methylation patterns, chromatin condensation, loss of acetylation of all four core histones by inhibition of histone acetyltransferase activity (HAT), increased levels of histone H3 lysine 9 dimethylation (H3K9me2) by inhibition of the activity of JHDM2A/JMJD1A, with no or very slight inhibition of G9a methyltransferase activity, increased histone ubiquitination by inhibition of histone deubiquitinating activity while having no effect on ubiquitinating activity, and increased levels of histone H3 Lysine 4 trimethylation (H3K4me3) [6–13]. Numerous studies have reported that arsenic disrupts global and gene-specific DNA methylation patterns, as well as, global levels of histone modifications [14, 15]. Arsenic has been shown to induce DNA hypomethylation of genes; such as, the metallothionein gene, and transcriptionally silence tumor suppressor genes by promoter hypermethylation [16–25]. *In vitro* exposure to arsenic was also shown to increase global levels of H3K9me2 and H3K4me3 and decrease global H3K27me3 [13, 26].

Until recently, the studies examining the changes in global levels of histone modifications induced by exposure to nickel or arsenic compounds had only been conducted in tissue culture model systems. Here, we review the first results reporting the effects of exposure to carcinogenic nickel or arsenic compounds on the global levels of histone modifications in the peripheral blood mononuclear cells (PBMCs) of a human subjects occupationally exposed to nickel compounds and subjects in Bangladesh exposed to arsenic in their drinking water.

Cases and methods

To determine the changes in global levels of H3K4me3 and H3K9me2 in PBMCs after *in vitro* exposure to nickel, 50 mL of blood was obtained from a healthy volunteer by venipuncture. Blood was collected by a registered nurse and PBMCs were isolated by Ficoll-Hypaque gradient procedure. PBMCs were exposed to NiCl₂ for 24 h at final concentrations of 0.25, 0.5, and 1.0 mM. To determine the changes in gene expression that occur in PBMCs after *in vitro* exposure to nickel, PBMCs isolated from a healthy volunteer were exposed to 0.25, 0.50, and 1.0 mM NiCl₂ for 24 h and were prepared for Affymetrix Human Genome U133A2.0 array containing 14,500 well-annotated genes. Sample preparation and analysis for ChIP-Seq with H3K4me3 have been previously described [27].

The nickel human study was conducted among workers of a nickel refinery in Jinchang, China, and referent subjects, local residents in Gansu, China. A total of 120 healthy male subjects between 24 and 56 years of age were recruited to this study; subjects with diagnosed chronic disease, including cancer, were excluded. Study site, subject recruitment, sample collection and handling, histone extraction, measurements of global histone modifications, urinary nickel, cotinine, and creatinine, and statistical analysis have been previously described [28]. Subjects with occupational exposure to nickel worked for at least 1 year in the flash smelting workshop of the nickel refinery where sulfidic nickel ores are

processed. Referent subjects, with no reported occupational exposure to nickel were either maintenance or office workers. For measurement of histone modifications in PBMCs of subjects, 30 subjects with high occupational exposure to nickel and 60 referent subjects were recruited. For measurement of intra- and inter-individual variance of global levels of histone modifications, 15 additional subjects with occupational exposure to nickel and 15 additional referent subjects were recruited. The study examining the global levels of histone modifications in PBMCs of subjects with occupational exposure to nickel and intra- and inter-individual variance in global levels of histone modifications in PBMCs has already been published [28]. To investigate if differential gene expression profiles occur in PBMCs of subjects with occupational exposure to high levels of nickel compared with referent subjects, a total of 10 subjects; 5 subjects with occupational exposure and 5 referent subjects were selected. Subjects with occupational exposure to ambient nickel concentrations as high as $1\text{mg}/\text{m}^3$. Referent subjects are exposed to ambient nickel concentrations of $204.8 \pm 268.6\text{ ng}/\text{m}^3$. No significant difference in age, self-reported data on smoking habits, and urinary cotinine were found between subjects with occupational exposure and referent subjects. Urinary nickel was elevated in subjects with occupational exposure to nickel ($p = 0.00004$).

The site for the arsenic study is located within Araihaazar, approximately 30 km east of Dhaka, Bangladesh, where a wide range of well-water arsenic concentrations ($0.1\text{--}960\text{ }\mu\text{g}/\text{L}$) are found in wells within this region. In a pilot study to evaluate the effects of arsenic exposure on histone modifications, a subset of 40 study participants, half male and half female, known to have a wide range of arsenic exposure were selected from the ongoing Folic Acid and Creatine Supplementation Trial (FACT). The FACT study participants are a subset of 600 of the HEALS cohort study, part of Columbia University's Superfund Research Program launched in 2000, that currently includes roughly 24,000 participants. Pregnant women or women planning a pregnancy were excluded from the FACT study. Participants currently taking nutritional supplements, or having known renal or gastrointestinal disease were also excluded. Informed consent was obtained by Bangladeshi field staff physicians. The study was approved by the institutional review boards of Columbia Presbyterian Medical Center and the Bangladesh Medical Research Council. PBMCs were isolated by Ficoll-Hypaque gradient procedure and global levels of histone modifications were measured as previously described [28].

Results and discussion

In vitro exposure of PBMCs to NiCl_2 for 24 h at final concentrations of 0.25, 0.5, and 1.0 mM induced an increase in global levels of H3K4me3 and H3K9me2 detected by both Western blot and ELISA methods (Figure 1 and Figure 2). To determine the changes in gene expression that occur in PBMCs after *in vitro* exposure to nickel, PBMCs isolated from a healthy volunteer, were treated with 0.25, 0.50, and 1.0 mM NiCl_2 for 24 h and prepared for Affymetrix Human Genome U133A2.0 array. Nickel-induced changes in gene expression displayed a dose-dependent response to treatment. First, the gene expression profiles between nickel-treated and untreated control sample were compared. A total of 1381 entities displayed a greater than 2-fold difference in expression in all treatments as compared to untreated control; of this subset, 40% of the entities were increased and 60% were decreased. When the cut-off threshold was increased to 3.5- and 5.0-fold difference, the numbers decreased to 382, and 246, respectively. Since H3K4me3 is a transcriptional activating mark that defines the location of the promoter region of genes and RNA transcripts, the location of H3K4me3 was mapped in the genome of PBMCs and among the set of identified nickel-induced genes in order to investigate the distribution of H3K4me3 after *in vitro* exposure to nickel using ChIP-Sequencing (ChIP-Seq) technology [27]. For this, PBMCs from a healthy volunteer were collected and treated with 0.5 mM NiCl_2 for 24 h or untreated control and ChIP-Seq was performed with an antibody specific for H3K4me3

on chromatin isolated from untreated and nickel-treated PBMCs. When the changes in gene expression were corroborated with the location of H3K4me3 mapped by Chip-Seq, it was determined that H3K4me3 mostly occurred at the transcriptional start site and the proximal promoter of genes and increases correlated with an increase in gene expression (Figure 3). Global H3K4me3 profiling of all nickel-induced genes in PBMCs with H3K4me3 peaks within 5,000 bp flanking the TSSs demonstrated two major peaks, a smaller pre-TSS peak and a larger post-TSS peak. After the pre-TSS peak, there was a significant dip in the signal at -100 (100 bp before the TSS), followed by a post-TSS peak which peaked at +400 (400 bp after TSS) [27]. These findings are consistent with a previous report that described the loss of nucleosomes and their assembly at the TSS of active genes [29]. Nickel treatment increased the height of the H3K4me3 peaks in addition to broadening of the H3K4me3 peaks globally in all nickel-induced genes. Whereas, previous studies have localized H3K4me3 to the promoter of genes, our results indicate that with nickel treatment this modification can extend great distances into the coding region of the nickel-induced genes. H3K4me3 extension into the coding region of genes induced by *in vitro* exposure to nickel has also been confirmed in the human lung adenocarcinoma A549 cell line [27].

H3K4me3 was found elevated ($p = 0.0004$) and H3K9me2 was found decreased ($p = 0.003$) in PBMCs of subjects with occupational exposure to high levels of nickel at a nickel refinery in China when compared to referent subjects (Table 1) [28]. H3K9ac did not vary between the two exposure groups ($p = 0.098$). In this same study, the intra-individual variance (variance within subjects) in comparison with inter-individual (variance between subjects) of global histone modifications was measured in order to determine if measurements of global histone modifications in PBMCs are fairly constant within subjects over time. The variations of H3K4me3, H3K9ac, and H3K9me2 were substantially larger between subjects relative to the variations within subjects in both groups, resulting in reliability coefficients (an estimate of the consistency of a set of measurements) of 0.60, 0.67, and 0.79 for H3K4me3, H3K9ac, and H3K9me2, respectively, for nickel-exposed subjects and 0.75, 0.74, and 0.97, respectively, for referent subjects (Table 2). These results suggest that global H3K4me3, H3K9ac, and H3K9me2 histone modifications are relatively stable over time in PBMCs from both nickel-exposed and referent subjects.

To date, no studies have examined the changes in gene expression profiles that may occur with occupational exposure to high levels of nickel. The gene expression profiles of PBMCs from both exposure groups were analyzed using the Affymetrix Human Gene 1.0 ST Array containing 28,869 well-annotated genes. The gene expression profiles of subjects with occupational exposure to nickel showed a clear separation from referent subjects (unpublished results). A total of 1646 genes in PBMCs from subjects with occupational exposure to high nickel levels displayed a greater than 1.25-fold change difference in all subjects when compared with the expression in PBMCs of referent subjects with low levels of nickel. The numbers of gene entities decreased to 312 and 35 when the cut-off threshold was increased to 1.50 and 2.0-fold difference, respectively (Figure 4A). The gene expression profiles in subjects with occupational exposure to high nickel levels clustered more closely with other subjects having a similar exposure compared to referent subjects. This separation was reported with Principal Component Analysis (PCA) of the microarray data (Figure 4B, red vs blue). A similar separation was observed with hierarchical clustering analysis of genes changed more than 1.5-fold in all subjects with occupational exposure, in which samples were sorted based on the similarity of gene expression (Figure 4C). Among nickel-induced genes, the largest group with respect to both degree of significance and number of genes was regulation of DNA binding and response to wounding in Biological Process. KEGG pathways overrepresented with nickel-induced genes were cytokine-cytokine receptor interaction and Graft-versus-host-disease. Among nickel-repressed genes, the largest group with respect to both degree of significance and number of genes was immune

response and defense response in Biological Process and KEGG pathways overrepresented with this list of genes were cytokine-cytokine receptor interaction and chemokine signaling pathway (unpublished results).

It has been previously shown that *in vitro* exposure of A549 cells to low doses of arsenic (1 μ M) increases global levels of H3K9me2 and decrease H3K27me3, both of which are associated with gene silencing, while increasing H3K4me3, was associated with gene activation [13, 26]. In a human population in Bangladesh where participants are exposed to arsenic in their drinking water, exposure to arsenic was associated with a significant, dose dependent, global increase in H3K9me2 ($p=0.004$) and a decrease and H3K9ac ($p=0.002$). Analyses of H3K4me3 and H3K27me3 and H3K27 acetylation levels revealed that, unlike H3K9me2 and H3K9ac, these marks differed in their response to arsenic exposure in a gender-specific manner (H3K27Ac ($p=0.049$), H3K27me3 ($p=0.048$), and H3K4me3 ($p=0.0081$)). H3K18ac also exhibited a gender specific response, but was not statistically significant.

Conclusions

Although the mechanisms for the carcinogenesis of nickel and arsenic compounds are unclear, experimental evidence suggest that these two metals affect gene expression via epigenetic mechanisms. *In vitro* exposure to nickel or arsenic has been shown to induce changes in global and gene-specific posttranslational histone modifications. In this review we describe the results of the first two studies examining the changes in global posttranslational histone modifications in PBMCs of human subjects exposed to these two metals. Elevated levels of H3K4me3 and decreased levels of H3K9me2 were found in subjects with occupational exposure to nickel at a nickel refinery in Jinchang, China. Subjects with exposure to arsenic in their drinking water were found to have elevated levels of H3K9me2 and decreased levels of H3K9ac. Interestingly, exposure to arsenic resulted in opposite changes in a number of histone modifications in males when compared with females. The results of these two studies provide evidence that alterations in posttranslational histone modifications do exist in PBMCs of human subjects with exposure to nickel or arsenic, and perhaps other carcinogenic metal compounds. Further identification of the specific genes whose gene expression is affected by exposure to these two metal compounds in these two populations is necessary. In the future, it would be necessary to conduct an analysis examining the changes in gene expression driven by the alterations in posttranslational histone modifications induced by exposure to either nickel or arsenic metals.

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Figure 1.
Increased global levels of H3K4me3 in PBMCs exposed to NiCl₂ *in vitro* for 24 h

Figure 2.
Increased global levels of H3K9me2 in PBMCs exposed to NiCl₂ *in vitro* for 24 h

Figure 3.
Genome distribution of H3K4me3 peaks in control and nickel-exposed PBMCs

Figure 4. Gene expression profiles of PBMCs of subjects with high occupational exposure to nickel compared with referent subjects. A. Number of genes differentially expressed between nickel-exposed subjects and referents. B. PCA analysis. C. Heat map analysis

Table 1

Increased global levels of H3K4me3 and decreased levels of H3K9me2 in PBMCs of subjects with occupational exposure to nickel.

	Referents mean $\pm$ SD	Occupational exposure mean $\pm$ SD	<i>p</i> -value
Histone modifications			
H3K4me3 (%)	0.15 $\pm$ 0.04	0.25 $\pm$ 0.11	0.0004
H3K9ac (%)	1.5 $\pm$ 0.36	1.28 $\pm$ 0.55	0.098
H3K9me2 (%)	0.15 $\pm$ 0.04	0.11 $\pm$ 0.05	0.003*
Urinary Ni (μ g/g creatinine)	4.0 $\pm$ 1.4	5.7 $\pm$ 1.9	0.0006
Age	42.3 $\pm$ 6.2	43.7 $\pm$ 4.1	0.26
Smoking (self-reported)			
Smokers [N/(%)]	[33/79%]	[19/83%]	0.26
Smoking years	17.0 $\pm$ 9.7	17.3 $\pm$ 6.8	0.57
Cigarettes/day	14.7 $\pm$ 9.6	18.1 $\pm$ 8.5	0.21
Urinary cotinine (μ g/g creatinine)	8401.5 $\pm$ 9440.9	11517.9 $\pm$ 10050	0.24

Table 2

Variance of global H3K4me3, H3K9ac, and H3K9me2 in PBMCs of subjects with environmental or occupational exposure to nickel.

	Variance between subjects	Variance within Subjects	Reliability coefficient (R) ^a
H3K4me3 (%)			
Referents	0.00003	0.00001	0.75
Occupational exposure	0.00003	0.00002	0.60
H3K9ac (%)			
Referents	14.6	5.3	0.74
Occupational exposure	37.0	14.9	0.67
H3K9me2 (%)			
Referents	0.005	0.0002	0.97
Occupational exposure	0.006	0.002	0.79