Gene Expression Profiles in Peripheral Blood Mononuclear Cells of Chinese Nickel Refinery Workers with High Exposures to Nickel and Control Subjects

Adriana Arita\textsuperscript{1}, Alexandra Muñoz\textsuperscript{1}, Yana Chervona\textsuperscript{1}, Jingping Niu\textsuperscript{2}, Qingshan Qu\textsuperscript{1}, Najuan Zhao\textsuperscript{2}, Ye Ruan\textsuperscript{2}, Kathrin Kiok\textsuperscript{1}, Thomas Kluz\textsuperscript{1}, Hong Sun\textsuperscript{1}, Hailey A. Clancy\textsuperscript{1}, Magdy Shamy\textsuperscript{3}, and Max Costa\textsuperscript{1}

Abstract

Background: Occupational exposure to nickel (Ni) is associated with an increased risk of lung and nasal cancers. Ni compounds exhibit weak mutagenic activity, alter the cell’s epigenetic homeostasis, and activate signaling pathways. However, changes in gene expression associated with Ni exposure have only been investigated \textit{in vitro}. This study was conducted in a Chinese population to determine whether occupational exposure to Ni was associated with differential gene expression profiles in the peripheral blood mononuclear cells (PBMC) of Ni-refinery workers when compared with referents.

Methods: Eight Ni-refinery workers and ten referents were selected. PBMC RNA was extracted and gene expression profiling was conducted using Affymetrix exon arrays. Differentially expressed genes (DEG) between both groups were identified in a global analysis.

Results: There were a total of 2,756 DEGs in the Ni-refinery workers relative to the referents [false discovery rate (FDR) adjusted \( P < 0.05 \)] with 770 upregulated genes and 1,986 downregulated genes. DNA repair and epigenetic genes were significantly overrepresented (\( P < 0.0002 \)) among the DEGs. Of 31 DNA repair genes, 29 were repressed in the Ni-refinery workers and 2 were overexpressed. Of the 16 epigenetic genes, 12 were repressed in the Ni-refinery workers and 4 were overexpressed.

Conclusions: The results of this study indicate that occupational exposure to Ni is associated with alterations in gene expression profiles in PBMCs of subjects.

Impact: Gene expression may be useful in identifying patterns of deregulation that precede clinical identification of Ni-induced cancers. Cancer Epidemiol Biomarkers Prev; 22(2); 261–9. ©2012 AACR.
chromatin structural alterations and changes in epigenetic marks have been implicated in Ni carcinogenesis (15–20). Previous studies have shown that in vitro exposure to Ni induces global DNA hypermethylation and can perturb global and gene specific levels of DNA methylation and posttranslational histone modifications. Ni-induced changes in posttranslational histone modifications can include loss of acetylation on histones H2A, H2B, H3, and H4, as well as increases in histone H3K9 dimethylation, H3K4 trimethylation, and H2A and H2B ubiquitylation (15–18).

The changes in gene expression patterns that occur in human populations occupationally exposed to Ni compounds have not been previously studied. Gene expression may be useful in identifying patterns of deregulation that precede clinical identification of Ni-induced cancers. Several studies have suggested that gene expression data from peripheral blood mononuclear cells (PBMCs) can provide valuable information about exposure levels (21–25). The use of PBMCs has tremendous potential in a therapeutic setting, as the use of blood as a surrogate for the primary tissue of interest greatly facilitates sample collection and analysis. Here, we have compared the differential gene expression profiles between subjects with occupational exposure to Ni (Ni-refinery workers) and referent subjects with low-level environmental exposure to Ni. The fact that the subjects from the high exposure group were local residents of the same providence, but with no known occupational exposure to Ni, provides a unique opportunity to identify gene expression changes associated with exposure to high levels of Ni.

**Materials and Methods**

**Study site and subject recruitment**

This study was conducted among Ni refinery workers in Jinchang, China, and local residents in Gansu, China. Jinchang City, where the Ni refinery is located, is within the providence of Gansu. Study selection was based on the fact that the subjects from the high exposure group were workers of the Ni refinery and the referent subjects were local residents of the same providence, but with no known occupational exposure to Ni. The human subject protocol for this study was approved by the Institutional Review Boards of both the New York University School of Medicine and the Lanzhou University School of Public Health. Written informed consent was obtained from all participating subjects.

The subjects for this study were randomly selected from a study population previously described (20). Eighteen healthy male subjects between 42 and 56 years of age were recruited; subjects diagnosed with chronic diseases, including cancer, were excluded. Eight subjects who had high occupational exposure to Ni having worked for at least 1 year at a Ni refinery in Jinchang, China in the flash smelting workshop where sulfidic Ni ores were processed and 10 referent subjects from residents in Gansu, China who were maintenance or office workers, with no reported occupational exposure to Ni, were also selected. Ni-refinery workers are exposed to ambient Ni concentrations as high as 1 mg/m³. Referent subjects are exposed to ambient air concentrations of 204.8 ± 268.6 ng/m³ of Ni. This study was conducted to determine whether Ni exposure was associated with differential gene expression profiles in the PBMCs of subjects.

**Sample collection and handling**

Blood collection, handling, and PBMC isolation for this study was conducted as previously described (20). Fifty milliliter of urine was also collected from each subject. The isolated PBMC pellet was suspended in TRIzol (Invitrogen) and urine samples were stored at −80°C and hand-carried frozen on dry ice to New York University.

**RNA isolation, amplification, and hybridization**

Total RNA was extracted from each sample according to the TRIzol (Invitrogen) manufacturer's protocol. cRNA probes were synthesized and labeled using GeneChip Whole Transcript Terminal Labeling Expression Kit (Affymetrix) and were subjected to hybridization with GeneChip Human Gene 1.0 ST Array (Affymetrix) that contains 28,869 well-annotated genes. Hybridization and scanning of the arrays were conducted using a standard procedure.

**Data analysis to identify differentially expressed genes**

Gene expression analyses were conducted using R. Gene expression data were imported and normalized in batches using the Affymetrix package version 1.30.0 in R 2.13.2 GUI 1.42 Leopard build 32-bit and robust multichip average (26, 27). Samples were then batch normalized and log-transformed using an empirical Bayes approach with the R package COMBAT run through SVA 3.0.2 in R 2.14.0 GUI 1.42 Leopard build 32-bit (28). Significance of gene expression changes between the exposed and referent populations was assessed using a gene-wise linear model approach with LIMMA 3.8.3, which uses an empirical Bayes approach to generate moderated t statistics by taking into account the standard errors and estimated log-fold changes (29). P values were subjected to FDR (false discovery rate) correction for multiple hypothesis testing and adjusted P values with P < 0.05 were considered significant (30). Genes that met significance thresholds via this approach were included in our study set for further analysis and are referred to as differentially expressed genes (DEG) or the “study set.” All microarray data is MIAME compliant and the raw data has been deposited in NCBI's Gene Expression Omnibus, and assigned Series accession number GSE40392.

To investigate the biologic relevance of the genes in our study set, we assessed the level of representation of previously established molecular signatures. Using the molecular signatures database (Broad Institute Gene Set Enrichment Analysis) the DEG were evaluated for overlap with gene sets related to position, biologic function, and disease states. This analysis led to the identification of a gene set involved in nasopharyngeal carcinoma (NPC) and further analysis was conducted using this...
experimentally generated set (31). DNA repair genes were also investigated based on their previously established link with NPC and were tested for their overrepresentation in the DEG set using a hypergeometric distribution as previously described (32). On the basis of this overlap, a list of 31 DNA repair genes were identified in the DEG and used in further analysis. Epigenetic genes were investigated based on Ni’s known role as an epigenetically active agent and were analyzed in the same manner as the DNA repair genes.

Heat maps and principal components analysis (PCA) plots were generated using R. The heat map in Fig. 1 was generated using the study set. The heat map in Fig. 2 was generated based on the intersection of the study set and genes downregulated in NPC (31). The heat map in Fig. 3A was generated by taking the intersection of the study set and an extensive list of DNA repair genes to generate a gene set of significantly changed DNA repair genes (33–35). The heat map in Fig. 3B was generated by taking the intersection of the study set and a compiled list of epigenetic related genes (36, 37). Correlation analysis for heat maps was conducted using Pearson correlation analysis and hierarchical clustering along both axes was conducted using the average method.

Real-time quantitative PCR

Total RNA was extracted from each sample using TRIzol Reagent (Invitrogen), and converted to single
stranded cDNA using Superscript III (Invitrogen). Quantitative real-time PCR (qRT-PCR) analysis was conducted using SYBR green PCR system (Applied Biosystems) on ABI prism 7900HT system (Applied Biosystems). All qRT-PCR reactions were conducted in triplicate. Because of lack of sufficient biologic material per sample, eight different RNA samples from the same study cohort that were not part of the original microarray analysis were used for the qRT-PCR analysis. Quantitative real-time PCR was conducted on CCL20, IL-6, and FOSB (upregulated Ni-refinery workers) and CX3CR1 and MNDa (downregulated in Ni-refinery workers) in four Ni-refinery workers and four referents. The data is presented as the absolute fold change in gene expression of Ni-refinery worker.

Figure 3. A, heat map of DNA repair genes. DNA repair genes include the following from top to bottom: XRCC5, CLK2, ATM, PRKDC, RAD50, PARP1, GTF2H3, RECQL, MRE11A, PRPF19, POLH, GTF2H1, MSH2, DDB1, UNG, PCNA, RPA2, PALB2, HLTf, DCLRE1A, TP53, APEX2, NHEJ1, FANCf, GTF2H4, RPA1, OGG1, MUS81, DDB2, PER1, and FEN1. See Supplementary Table S3 for the list of genes and associated P values. B, heat map of epigenetic genes. Epigenetic genes include the following from top to bottom: HDAC2, EHMT1, DMNT1, SETDB1, SETDB2, MYST1, PMRT7, HDAC1, PRMT5, HDAC8, TAF1, EHMT2, MYST2, EZH2, JMJD6, and PRMD5. See Supplementary Table S4 for the list of genes and associated P values.
samples relative to the referents. The expression of the target genes was first normalized to the expression of the house-keeping gene (GADPH) for the referent and Ni-refinery worker samples. Then the mean of the Ni-refinery worker samples was normalized to the mean of the referent samples.

Measurement of urinary nickel, cotinine, and creatinine
Urinary Ni was used to index the individual personal exposure to Ni and was analyzed for all study subjects by inductively coupled plasma mass spectroscopy (Elan DRCII, Perkin Elmer; ref. 38). Urinary cotinine, a major metabolite of nicotine and a valid biomarker of environmental tobacco smoke, was measured in each subject to confirm smoking status and control its potential confounding effects. Urinary cotinine was measured using a cotinine direct ELISA kit (Immunalysis; ref. 39). Urinary creatinine was measured to adjust the Ni and cotinine levels in urine samples. Urinary creatinines were determined using the creatinine incorporating dynamic stabilization technology assay kits (Fisher Scientific) according to the standard procedure.

Statistical analysis
Differences in age, self-reported smoking data (number of smokers, number of years smoking, and cigarettes per day smoked for smokers), urinary cotinine, and urinary Ni among groups were compared by 2-sample Student’s t test. Differences in number of smokers and nonsmokers between exposure groups were compared by \( \chi^2 \) test. All \( P \) values were 2 sided, with \( P < 0.05 \) considered statistically significant. Statistical analyses were conducted in S-Plus statistical analysis software (TIBCO Software Inc.).

Table 1. Characteristics of the Ni-refinery workers and referents selected to assess gene expression profiles in PBMCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Referent subjects</th>
<th>Ni-refinery workers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age</td>
<td>43.90 ± 8.84</td>
<td>45.50 ± 5.45</td>
</tr>
<tr>
<td>Smoking (self-reported)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers [N/(%)]</td>
<td>[7/70%]</td>
<td>[6/75%]</td>
</tr>
<tr>
<td>Smoking years</td>
<td>10.70 ± 10.91</td>
<td>12.75 ± 10.69</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>11.70 ± 15.35</td>
<td>9.25 ± 9.44</td>
</tr>
<tr>
<td>Urinary cotinine (µg/g creatinine)</td>
<td>8.115.82 ± 10,345.48</td>
<td>9,034.08 ± 13,552.52</td>
</tr>
<tr>
<td>Urinary Ni (µg/g creatinine)</td>
<td>3.41 ± 3.30</td>
<td>6.86 ± 1.60</td>
</tr>
<tr>
<td>Median</td>
<td>2.37</td>
<td>3.32</td>
</tr>
<tr>
<td>Min</td>
<td>1.19</td>
<td>2.23</td>
</tr>
<tr>
<td>Max</td>
<td>12.58</td>
<td>9.44</td>
</tr>
<tr>
<td>10th percentile</td>
<td>1.19</td>
<td>5.23</td>
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<tr>
<td>25th percentile</td>
<td>2.30</td>
<td>5.37</td>
</tr>
<tr>
<td>75th percentile</td>
<td>3.25</td>
<td>8.38</td>
</tr>
<tr>
<td>90th percentile</td>
<td>12.58</td>
<td>8.38</td>
</tr>
</tbody>
</table>
Table 2. Validation of microarray results by quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
<th>Microarray</th>
<th>qRT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemokine (C-C motif) ligand 20</td>
<td>CCL20</td>
<td>4.20</td>
<td>8.79</td>
</tr>
<tr>
<td>Interleukin-6 (interferon, beta 2)</td>
<td>IL6</td>
<td>4.02</td>
<td>4.8</td>
</tr>
<tr>
<td>FBJ murine osteosarcoma viral oncogene homolog B</td>
<td>FOSE</td>
<td>2.14</td>
<td>1.75</td>
</tr>
<tr>
<td>Chemokine (C-X3-C motif) receptor 1</td>
<td>CX3CR1</td>
<td>0.26</td>
<td>0.51</td>
</tr>
<tr>
<td>Myeloid cell nuclear differentiation antigen</td>
<td>MNDKA</td>
<td>0.35</td>
<td>0.40</td>
</tr>
</tbody>
</table>

NOTE: Data is represented as absolute fold-change values for the exposed group (Ni-refinery workers) relative to the referents for both the microarray and qRT-PCR.

Table 3. Gene families represented with list of genes differentially expressed between Ni-refinery workers and referents ($P < 0.05$)

<table>
<thead>
<tr>
<th>Gene family</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogenes</td>
<td>46</td>
</tr>
<tr>
<td>Tumor suppressors</td>
<td>21</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>233</td>
</tr>
<tr>
<td>Cytokines/growth factors</td>
<td>37</td>
</tr>
<tr>
<td>Protein kinases</td>
<td>98</td>
</tr>
<tr>
<td>Translocated cancer genes</td>
<td>46</td>
</tr>
<tr>
<td>Cell differentiation markers</td>
<td>82</td>
</tr>
<tr>
<td>Homeodomain proteins</td>
<td>12</td>
</tr>
</tbody>
</table>

qRT-PCR was conducted in four Ni-refinery workers and four referents on CCL20, IL-6, FOSE, (upregulated in Ni-refinery workers), CX3CR1 and MNDKA (downregulated in Ni-refinery workers). As shown in Table 2, the up- or downregulated patterns for these 4 genes obtained from qRT-PCR were similar to those in the microarray study. In comparing the fold-change values between the microarray data and the qRT-PCR data (Table 2), we see the changes are comparable in that both sets of data reflect fold changes occurring in the same direction with similar magnitudes. It is important to note that this comparison is occurring across different types of data, where the fold-change values for the microarray data are qualitative and the fold-change values for the qRT-PCR are quantitative, and therefore, we do not expect such values to be exactly the same. Thus, the match in direction and the similarity in magnitude constitute consistency in the findings.

To assess the biologic relevance of the DEG, (adjusted $P < 0.05$) those genes exhibiting more than a 1.15 fold-change difference in gene expression (Supplementary Table S1), including a total of 2,734 genes, with 756 upregulated and 1,978 downregulated genes, were identified as differentially expressed between Ni-refinery workers and referents, where the left subbranch (A) was characterized largely by upregulation and the right subbranch (B) was characterized by expression levels near the mean (Fig. 3A). Epigenetic genes were also significantly overrepresented in the DEG by 2-fold ($P < 0.0002$). Of 31 DNA repair genes, 29 were repressed in the Ni-refinery workers and 2 were overexpressed (Supplementary Table S3). Cluster analysis with DNA repair genes yielded 2 primary clusters that largely correspond to the occupational and referent populations, but unlike the cluster formation in the other study sets, there were 2 main subbranch clusters within the referent population, where the left subbranch (A) was characterized largely by overexpression and the right subbranch (B) was characterized by expression levels near the mean (Fig. 3A). Epigenetic genes were also significantly overrepresented in the DEG by 2-fold ($P < 0.02$). Of these 16 epigenetic genes, 12 were repressed in the Ni-refinery workers and 4 were overexpressed (Supplementary Table S4). Similar results were observed with a hierarchical clustering analysis of epigenetic genes in which samples were sorted based on the similarity of gene expression (Fig. 3B).

Discussion

Ni is a well-established human carcinogen associated with an increased risk of lung and nasal cancers. However, the molecular mechanisms and changes in gene expression that mediate Ni’s toxicity and carcinogenicity are not well understood. In the present study, we found that differential patterns of gene expression in PBMC exist among Ni-refinery workers compared with referent...
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subjects, who are only environmentally exposed to Ni. There were a total of 2,756 DEGs in the Ni-refinery workers relative to the control subjects (FDR adjusted \( P < 0.05 \)) with 770 upregulated genes and 1,986 downregulated genes. Although various mechanisms for the carcinogenesis of Ni compounds have been postulated, the changes in gene expression associated with occupational exposure to Ni have not been previously investigated. Here we have found that the function of the majority of the Ni-induced and repressed genes by in vivo exposure to Ni were related to cytokine–cytokine interaction and chemokine signaling (IL1A, CCL20, CCR2, CX3CR1, IL6, IL1RN, TNFSF10, IL8RB, and IL8RA). Of particular interest is IL-1, a cytokine known to be elevated in many tumor types and which has been implicated as a factor in tumor progression by its ability to increase the expression of metastatic, angiogenic, and growth factor genes (41). Interestingly, IL1A was the most upregulated gene in the PBMCs of the Ni-refinery workers. These results suggest that high exposure to Ni compounds was associated with increased expression of cancer related genes. In this case, the high expression of IL1A and other genes in Ni-refinery workers suggests that these genes should be further studied to identify their role in Ni-induced adverse health effects.

Moreover, the genes aberrantly expressed in this study share a significant similarity with those aberrantly expressed in NPC tumors as described in Dodd et al. (2006). Nasal and sino-nasal cancers have been historically established as an endpoint of occupational Ni exposure (2, 3), and the similarity in gene deregulation between the refinery workers in our study and NPC tumors, suggests that gene expression profiling can be used to link human in vivo Ni exposures to cancerous endpoints by identifying gene expression patterns that precede clinical presentations of cancer. Although the two cancer endpoints in discussion here are not synonymous, their anatomical proximity and the significance of our \( P \) value merits further consideration. While genes from NPC were significantly overrepresented in our study, there was not a complete overlap and the direction of deregulation was only partially consistent with the Dodd study set. These results likely reflect the similar endpoints, cancers of the nasal and nasopharynx regions, but also point toward the different etiologies likely involved in these related but distinct cancers. The Dodd study emphasizes that NPC is often associated with Epstein-Barr virus and that factor is not one that we would expect to be represented in our study. Both etiologies show a strong involvement of the DNA repair genes, but involve different groups of genes with only UNG, MSH2, PRKDC, and PCNA in common. The Dodd and colleagues’ study found DNA repair genes to be upregulated, whereas we observed that DNA repair genes were largely repressed in the exposed population. The repression of DNA repair genes observed in our study may be explained by Ni’s role as an epigenetically active agent that can actively silence genes. The repression of DNA repair genes was associated with high levels of exposure, and may be due to Ni-induced silencing, a process which is supported by the significant overrepresentation of epigenetic genes in the study set, their aberrant expression patterns relative to the referents, and a previous study which showed that occupational exposure to Ni induces consistent changes in histone modifications (15–18, 20, 42). Together, the changes observed in epigenetic regulation and suppression of DNA repair can be detrimental to the genetic stability of cells, lead to increased spontaneous mutations, and promote conditions in which carcinogenesis is favored.

The importance of identifying genes through an in vivo format can be highlighted with a comparison of our in vivo data and pilot data from an in vitro study. We assessed the short-term gene changes associated with PBMC’s, exposed to Ni in vitro and found that gene changes at the 1.5 fold-change level were strikingly different from those observed in the in vivo study. PBMC’s were exposed to Ni chloride (NiCl2) at 1.0, 0.5, and 0.25 mmol/L for 24 hours and single samples were then prepared for gene expression analysis and hybridized to Affymetrix HGU133a.2 arrays. For all 3 treatments, there were 98 genes that changed 1.5 fold (Supplementary Table S5). Of these genes, only 9 were found among the genes that changed 1.5-fold in the in vivo study. These genes include: CD14, FUCAI, DAPK1, HMOX1, FGL2, IGF6, FCGR1B, TLRA, and CLEC7A. While the data from the in vitro study remains preliminary and was not subjected to statistical analysis due to a lack of replicates, it does suggest that in vitro exposures do not accurately replicate the gene expression changes found in vivo. Furthermore, the gene changes observed in vitro do not share the similarity with NPC that was evident in vivo, again indicating the importance of conducting in vivo human studies to identify biomarkers that are linked with disease endpoints.

Identification of genes associated with high occupational exposure to Ni will help identify agents that combat the toxic effects of exposure to Ni or can be used as biomarkers for Ni exposure and effects. It is of interest that occupational exposure to Ni causes 29 of 31 DNA repair genes to be suppressed in expression, whereas in the referent group these same genes were upregulated in the left subbranch (Fig. 3A, Branch A) and were expressed near the mean in the right subbranch (Fig. 3A, Branch B). Interestingly, the repression of 6 of the DNA repair genes (MUS81, PALB2, XRCC5, PRKDC, CLK2, HLF, and TP53) found in this study were also found suppressed in the Beas-2B cell line exposed to Ni in vitro (43). This same study also found the expression of 6 epigenetic genes (EHMT1, EHMT2, PRMT7, DEDD1, HLF, and JMD6) repressed as in our study (43). The formation of three primary clusters and 3 types of DNA repair response suggests the presence of a Ni-induced dose-response mechanism. In subjects with occupational exposure to Ni, DNA repair genes were greatly repressed, and in the referent population there were 2 types of low-level responses, in the first response type the DNA repair genes were highly upregulated (Fig. 3A, Branch A), whereas in the second there was no distinct response with values near
the mean (Fig. 3B, Branch B). This clear difference between the worker group and branch A of the referent population suggests there may be a dose–response mechanism whereby high exposure leads to the repression of DNA repair genes and low exposure can lead to their over-expression. While the urinary Ni levels for these 2 groups (worker group and branch A of referents) are not significantly different, the means for the 2 groups do reflect this trend. Further study is needed to determine whether the 2 types of low-level response for branches A and B of the referent population are reflective of a dose–response mechanism or of another factor such as a genetic polymorphism as there was no statistically significant difference in the urinary Ni levels for these 2 groups either. However, it should be noted that urinary Ni may not be a true measure of worker exposure to Ni, as inhaled particulate Ni compounds (i.e., Ni oxides, etc.) may not be cleared from the lungs into the urine for months to years (44, 45). Although Ni-exposure resulted in the differential expression of a large number of genes, these DEGs may not only be Ni-response genes. Future experiments should focus on whether the gene expression changes found in this study are specific to Ni exposure and/or toxicity and determine whether changes are occurring as a result of exposures to other agents found in Ni refineries.

In summary, we have identified many novel changes in gene expression that were different from the immediate response genes identified from previous studies using acute in vitro exposure of tissue culture cells to Ni. These genes may be involved in Ni-induced toxicity, carcinogenesis, and can be used as potential biomarkers of exposure to Ni compounds. Furthermore, these changes also show strong similarity with changes observed in NPC, suggesting that biomarkers of disease may be developed from gene expression studies of PBMCs. Thus, further studies of the mechanism(s) of Ni-induced alterations in gene expression patterns may be a useful tool for identifying patterns of deregulation that precede clinical identification of Ni-induced cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A. Arita, Y. Chervona, Q. Qu, M. Shamy, M. Costa
Development of methodology: A. Arita, H. Sun
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Arita, J. Niu, Q. Qu, N. Zhao, Y. Ruan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Arita, A. Muñoz, Y. Chervona, Q. Qu, H. A. Clancy, M. Shamy
Writing, review, and/or revision of the manuscript: A. Arita, A. Muñoz, Y. Chervona, M. Shamy, M. Costa
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Arita, A. Muñoz, T. Kluz, M. Costa
Study supervision: A. Arita, M. Costa
PCR and qPCR: K. Kook

Grant Support
This work was supported by grant numbers ES00260, ES01034, ES014454, ES005512, and 5 T32 ES07024 from the National Institutes of Environmental Health Sciences, and support from grant number CA16087 from National Cancer Institute and RR029893 from National Center for Research Resources.

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doi:10.1158/1055-9965.EPI-12-1011

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