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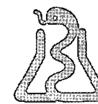

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Early Gene Expression in Mouse Spleen Cells after Exposure to Nickel Acetate

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ABSTRACT. Exposure to soluble nickel compound produces toxic effects on immune system, but the mechanism of action remains to be elucidated. Differential gene expression was studied to understand the potential molecular mechanism responsible for acute toxicity induced by nickel acetate in spleen cells. We exposed mouse spleen cells to nickel acetate with a nontoxic dose (40 μM) and then extracted total RNA at 6 h and 12 h after exposure. The RNA was hybridized onto 10K mouse oligonucleotide microarrays, and data were analyzed using GeneSpring 7.1. Nickel had a modest effects on expression of many genes, in the range of 1.3~3 fold. The expression profile showed time-dependent changes in expression levels of differentially expressed genes, including some important genes related to cell cycle, apoptosis and DNA repair. In hierarchical cluster analysis of duplicate experiments, 111 genes were screened out. Out of these, 44 genes showing time-dependent up-regulation (>1.5 fold) and 38 genes showing down-regulation (<1.5 fold) at all time points were chosen for further analysis. The change in the expression of three genes (*GPX1*, *GADD45B* and *FAIM*) after nickel treatment was validated using RT-PCR. As a rule, a number of genes appear to be coordinately regulated between cell survival and cell death from nickel toxicity. In conclusion, changes in the gene profile in the spleen after nickel treatment are complex and genes with diverse functions are modulated. These findings will be contributed to the understanding of the complicated biological effects of nickel.

Keywords: Nickel, Microarray, Apoptosis, Oligochip, Gene expression, Spleen.

INTRODUCTION

Nickel is known to produce a variety of health hazards in human and experimental animals due to its ability to induce toxic effects in various organs and tissues, following either acute and chronic exposure (Oller *et al.*, 1997; Haber *et al.*, 2000). Nickel, combined with other elements, occurs naturally in earth crust. However, exposure to this toxic heavy metal is usually associated with nickel refining processes (calcination, smelting, roasting, and electrolysis) and from nickel plating and polishing operations (electrolysis and grinding). Epidemiological data in industrially exposed humans suggest that it causes dermatitis and lung and sinonasal cancers (Kasprzak *et al.*, 1990).

It is also known that nickel affects the transcription of a number of genes. Nickel induces the genes that have protective functions, including those coding for H ferritin and metallothionein IE that chelate nickel(II) to make it biologically inert, heat shock proteins that play roles in renaturing damaged proteins and *SH3BGRL3* that plays an anti-oxidative role (Lee *et al.*, 1999; Cheng *et al.*, 2003; Koh and Lee, 2004; Kowara *et al.*, 2005).

Some of protooncogenes, including *c-myc* and *jun* that might be related to the mechanistic background of carcinogenesis, also were over-expressed in murine fibroblasts transformed by nickel (Kowara *et al.*, 2005). Changes in the expression of other proteins were noted

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as well. These include inactivation of senescence genes (Klein and Costa, 1997), antiangiogenetic thrombospondin gene (Salnikow *et al.*, 1997), silencing of a telomere marker gene (Broday *et al.*, 1999), induction of hypoxia-regulated gene *cap43* (Zhou *et al.*, 1998), *NF-kB* (Huang *et al.*, 2002), and *HIF-1* transcription factor gene (Salnikow *et al.*, 2003). The advent of the microarray technique allowed for much broader analysis of nickel influence on gene expression. However, these effects of nickel on gene expression probably represent only a fraction, and there might be a number of unidentified effects on gene relevant to the expression of toxicity and protection against it.

Exposure to nickel compounds also produces several immunologic effects, including stimulation of inflammation (Zhong *et al.*, 1990), thymic involution, decreased spleen T cell number, and decreased proliferative responses to T cell mitogens in mice (Smialowicz *et al.*, 1985; Kasprzak *et al.*, 1987), as well as significant declines in natural killer cells activity in mice and rats (Smialowicz *et al.*, 1984; Kasprzak *et al.*, 1988). Previous research with murine T cell hybridoma cells showed that cell death induced by nickel was associated with increased Fas ligand expression (Kim *et al.*, 2002). However, the effect of nickel exposure on immune system is still unclear. So, altered mRNA expression in nickel-treated mouse spleen cells is of fundamental importance for the understanding of molecular basis of nickel toxicity on immune cells. The present study used a dose of nickel that does not induce overt toxicity in mouse spleen cells to examine effects on gene expression using microarray. These results indicate that nickel exposure induces significant changes in gene expression in the spleen, and detect several genes that might have important roles in the nickel-induced toxicity and protection against it.

MATERIALS AND METHODS

Chemicals

Nickel acetate was purchased from J.T. Baker (Phillipsburg, NJ. USA), the 10K oligochip was from Genomictree (Daeduck, Korea), Trizol Reagent was from Life Technologies Inc. (Gaithersburg, MD, USA) and Quantum RNA RT-PCR kit was from Ambion (Austin TX, USA).

Cell culture and nickel treatment

Five-week-old male ICR mice were purchased from Samtaco Bio Korea (Osan, Korea) and were acclimated for 7 days in pathogen-free conditions before treatment. The animals were housed in polycarbonate cages and given food and water *ad libitum*. To minimize the possible effects of individual variations, spleen cells were isolated from one spleen tissue of each of eight mice per group and pooled. Pooled spleen cells were cultured at 37°C in humidified atmosphere containing 5% CO_2 in RPMI 1640 medium supplemented with 1% fetal bovine serum, 1 mM glutamine, 100 units of penicillin/ml and 100 μg of streptomycin/ml. At 2 h before the addition of nickel acetate, 1×10^7 cells/plate were seeded in 15 cm^2 culture plate. Cells were cultured in the presence or absence of nickel acetate (40 μM) for 12 h and then harvested for microarray analysis. For toxicity tests, 3×10^5 cells were incubated for 12 h with 0~320 μM nickel acetate, washed with PBS, and stained with trypan blue, and the number of damaged cells counted.

Microarray analysis

Total spleen RNA was prepared from untreated and nickel-treated cells using Trizol reagent and was inspected to be free of degradation by agarose gel electrophoresis. With these samples, oligonucleotide microarrays containing 10,000 mouse genes were done following the manufacture's instructions. Briefly, mRNAs from untreated and nickel-treated cells were reverse-transcribed into cDNAs, and were simultaneously labeled with fluorescent dyes Cy3 and Cy5, respectively. Both the Cy3 and Cy5-labeled cDNA were purified using PCR purification kit (Qiagen Co, Hilden, Germany). The purified cDNA was resuspended in 100 ul of hybridization solution containing 5× SSC, 0.1% SDS, 30% formamide, 20 μg of Human Cot-1 DNA, 20 μg of poly A RNA and 20 μg of Yeast tRNA (Invitrogen, Carlsbad, CA, USA). The hybridization mixtures were heated at 100°C for 2~3 min and directly pipetted onto microarrays. The arrays hybridized at 42°C for 12 h in the humidified hybridization chamber. The hybridized microarrays were washed with 2× SSC/0.1% SDS for 5 min, 0.1× SSC/0.1% SDS for 10 min, and 0.1× SSC for 2 min two times. The washed microarrays were immediately dried using the microarray centrifuge.

Data acquisition and analysis

The hybridization images were analyzed by GenePix-Pro 4.0 (Axon Instruments, CA, USA). The average fluorescence intensity for each spot was calculated and local background was subtracted. All data normalization and selection of fold-changed genes were performed using GeneSpring 7.1 (Silicon Genetics, USA). The reliable genes were filtered with cut-off value based on two component error model after intensity-dependent normalization (LOWESS). The averages of normalized ratios were calculated by dividing the average of

normalized signal channel intensity by the average of normalized control channel intensity. The ANOVA test (parametric) and single t test were performed at the p values <0.01 or 0.05 to find genes that differentially expressed across conditions. Two hybridization replicates were carried out for each sample, the mean and the SD of hybridization intensity were calculated for each gene at each time point, and the expression ratios of nickel(II)-treated over controls were determined. Genes that met the following criteria were chosen for further analysis: (1) hybridization intensity >3000; (2) ratios greater than 1.3-fold (up-regulated) or less than 0.77-fold (down-regulated); (3) statistically significant (P<0.05). Functional annotation of genes was performed according to Gene OntologyTM Consortium (http://www.geneontology.org/index.shtml) by GeneSpring 7.1.

RT-PCR

Total RNA from the Trizol isolate was treated with RNase-free DNase I. After removal of the DNase I, 1 ug of total RNA were reverse-transcribed using random hexadeoxynucleotide primer. The genes of interest (GPX, *FAIM*, and *GADD45*) and the housekeeping gene β-actin were analyzed by Quantum RNA RT-PCR kit according to the manufacturer's protocol (Ambion, Austin TX, USA). The following primers were used to amplify *GPX1*: forward 5'-CCTCAAGTACGTCCGACCTG-3' and reverse 5'-CAATGTCGTTGCGGCACACC-3' (197-bp), *FAIM*: forward 5'-GAGAGCTGCTGACTACGTCG-3' and reverse 5'-GACCATTGCACCATACGTCC-3' (447-bp), *GADD45B*: forward 5'-CCCTCATCCCCCAGAACAATC-3' and reverse 5'-TCGCCCTCCGCTGACTTATG-3' (332-bp). β-actin primers (Ambion) were used as an internal standard (294-bp). PCR was performed at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s for 33 cycles, followed by a final elongation for 7 min. PCR products were electrophoresed on 2% agarose gel, and visualized by ethidium bromide staining.

RESULTS

When cells were incubated with nickel-containing media for 12 h, no significant effects on the cells were noted up to 40 μM, while concentrations above 160 uM resulted in increase of the damaged cells. The 40 μM concentration was selected for gene expression studies; this concentration was considered as nontoxic dose (Fig. 1).

Total RNA was prepared from untreated and nickel-treated mouse spleen cells. Two hybridization replicates were carried out for each sample, the mean of hybridization intensity were calculated for each gene at each time point, and the expression ratios of nickel-treated over controls were determined. Correlation coefficients between two replicates at 6 h and 12 h were 0.952 and 0.973, respectively (Fig. 2). These were highly reproducible. The scatter plots for two sets of nickel-treated/control spleen cell cultures were apparent that few genes showed marked difference in expression, as expected from the low nickel dose and short-term treatment (Data not shown). So, we opted for a strategy involving multiple criteria to identify potentially significant changes in cDNA levels. Genes that met the following criteria were chosen for further analysis: (1) hybridization intensity 3000; (2) ratios greater than 1.3-fold (up-regulated) or less than 0.77 (down-regulated); (3) statistically significant (P<0.05).

Hierarchical cluster analysis was used to profile gene expression patterns in response to nickel acetate treatment in the spleen. A total 82 genes showing time-dependent change belong to two major groups. Forty-four genes in the group 1 show an time-dependent up-regulation greater than 1.5-fold at 12 h, compared to the result at 6 h (Table 1). Among the up-regulated genes

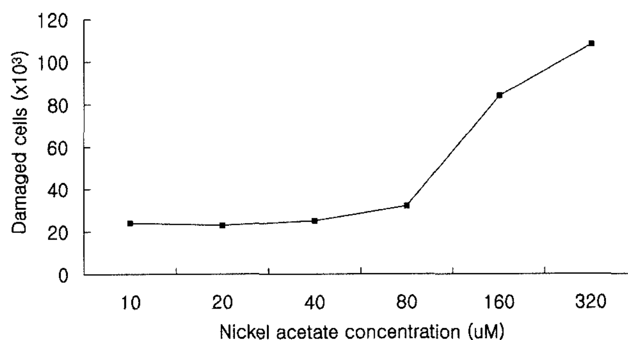

Fig. 1. Dose response of acute cytotoxicity in mouse spleen cells treated with nickel acetate for 12 h. Values are the mean of two independent experiments.

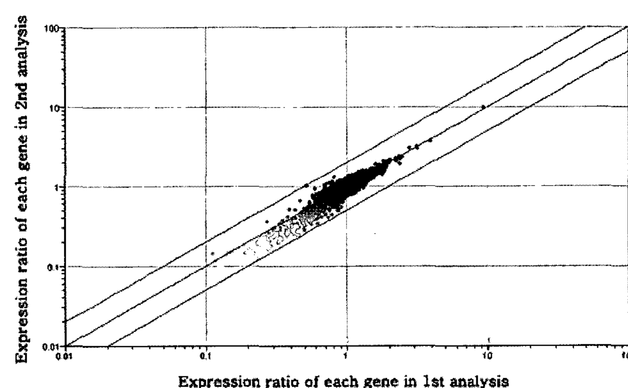

Fig. 2. Correlation analysis of the cDNA expression data for two independent microarrays of mouse spleen cells treated with nickel acetate for 6 h vs. control.

Table 1. Expression profiles of genes showing time-dependent up-regulation >1.5-fold at 12 h, compared to the result at 6 h in mouse spleen cells treated with nickel acetate.

GeneBank ID	Gene Name	Function	6 h	12 h
AK020624	*SOD1*, Superoxide dismutase 1	Removal of superoxide radical	0.799	1.374
NM_00757	*BTG2*, B-cell translocation gene 2	DNA repair	1.165	1.848
NM_00816	*GPX1*, Glutathione peroxidase 1	Induction of apoptosis by oxidative stress	1.088	1.500
NM_-01974	*PDCD5*, Programmed cell death 5	Induction of apoptosis	1.017	1.327
NM_0188	*PLAGL2*, Pleimorphic adenoma gene-like 2	Induction of apoptosis	0.925	1.538
NM_00856	*MCL1*, Myeloid cell leukemia sequence 1	Anti-apoptosis	0.808	1.340
NM_00942	*TPT1*, Tumor protein, translationally-controlled 1	Anti-apoptosis	0.878	1.707
NM_02538	*ANAPC11*, Anaphase promoting complex subunit 11 homolog	Negative regulation of apoptosis	1.034	1.669
AK003861	*TGFBR2*, Transforming growth factor, beta receptor II	Regulation of cell proliferation	0.878	1.707
NM_00865	*GADD45B*, Growth arrest and DNA damage -inducible 45 beta	Regulation of cell cycle	0.933	1.471
NM_01079	*MIF*, Macrophage migration inhibitory factor	Regulation of cell proliferation	0.865	1.364
NM_00785	*CD53*, CD53 antigen	Regulation of growth	1.167	1.623
NM_01079	*LEF1*, Lymphoid enhancer binding factor 1	Cell development	1.102	1.675
NM_0109	*NFKBA*, Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	Negative regulation of Notch signaling pathway	1.011	1.835
NM_00765	*CD79A*, CD79A antigen	B cell receptor signaling pathway	1.012	1.357
NM_00986	*CDC42*, Cell division cycle 42 homolog	Signal transduction	0.931	1.464
NM_0101	*EDG6*, Endothelial differentiation, G protein-coupled receptor 6	G-protein signaling	1.079	1.981
NM_02445	*PDC*, Phosducin	Regulation of G-protein coupled receptor protein signaling	1.040	1.581
NM_02612	Src-like adaptor 2	T-cell activation	0.894	1.684
NM_05283	*RPL10*, Ribosomal protein 10	Protein synthesis	0.960	1.570
NM_00907	*RPL12*, Ribosomal protein L12	Protein synthesis	0.834	1.456
NM_00907	*RPL22*, Ribosomal protein L22	Protein synthesis	0.822	1.836
NM_00908	*RPL26*, Ribosomal protein L26	Protein synthesis	0.902	1.642
NM_01128	*RPL27*, Ribosomal protein L27	Protein synthesis	0.864	1.367
NM_01376	*RPL3*, Ribosomal protein L3	Protein synthesis	0.905	1.599
NM_00908	*RPL37A*, Ribosomal protein L37a	Protein synthesis	0.875	1.406
NM_01205	*RPL8*, Ribosomal protein L8	Protein synthesis	0.867	1.418
NM_02653	*RPL13*, Ribosomal protein S13	Protein synthesis	0.830	1.583
NM_00909	*RPL17*, Ribosomal protein S17	Protein synthesis	0.865	1.368
NM_02427	*RPL27A*, Ribosomal protein S27a	Protein synthesis	0.853	1.294
NM_00909	*RPS6*, Ribosomal protein S6	Protein ser/thr kinase activity	0.878	1.637
NM_0113	*RPS7*, Ribosomal protein S7	Protein synthesis	0.840	1.500
AK004814	*QARS*, Glutamyl-tRNA synthetase	Protein synthesis	0.758	1.353
NM_03246	*CD96*, CD96 antigen	Integral protein	1.059	1.584
NM_0168	*RHOA*, Ras homolog gene family, member A	cell-matrix adhesion	0.010	1.435
NM_01993	*CXCL4*, Chemokine (C-X-C motif) ligand 4	Chemotaxis	0.936	1.989
NM_01166	*UBE2I*, Ubiquitin-conjugating enzyme E2I	Protein catabolism	0.915	1.658
NM_01671	*CUL3*, Culin 3	Protein ubiquitination	0.868	1.445
AK002769	*GLPC*, Glycophorin C	Protein glycosylation	1.006	1.522
NM_02052	*SLC25A20*, Solute Carrier family 25, member 20	Transport	0.838	1.646
NM_02957	*TXNDC4*, Thioredoxin domain containing 4	Electron transport	0.902	1.456
NM_00994	COX7A2, Cytochrome oxidase subunit VIIa 2	Electron transport	0.867	1.769
AK016981	*TLOC1*, Translocation protein 1	Protein transport	0.857	1.312
NM_02151	*RAB2*, RAB2, member RAS oncogene family	Intracellular protein transport	0.836	1.610

there were five cell growth regulation genes (*TGFBR2*, *GADD45B*, *MIF*, *CD53*, and *LEF1*), three pro-apoptotic genes (*PDCD5*, *GPX1*, and *PLAGL2*), three anti-apoptotic genes (*MCL1*, *TPT1*, and *ANAPC11*), and two genes involved in protecting the cells from DNA damage (*SOD1* and *BTG2*). Thirty-eight genes in the group 2 show an down-regulation greater than 1.5-fold at 6 h and 12 h (Table 2). These genes include pro-apoptotic gene (*GZMA*), anti-apoptotic gene (*FAIM*), and cell growth regulation genes (*E2F5*). Gene profiles of these

Table 2. Expression profiles of genes showing down-regulation >1.5-fold at 6 h and 12 h in mouse spleen cells treated with nickel acetate

GeneBank ID	Gene Name	Function	6 h	12 h
NM_01037	*GZMA*, Granzyme A	Apoptosis	0.488	0.507
AK013476	*FAIM2*, Fas apoptotic inhibitory molecule 2	Anti-apoptosis	0.221	0.136
NM_00789	*E2F5*, E2F transcription factor 5	Regulation of cell cycle	0.415	0.559
NM_00875	*DEFCR5*, Defensin related cryptdin 5	Defense	0.180	0.262
NM_02833	*ANGPTL1*, Angiopoietin-like 1	Protein tyrosine kinase signaling pathway	0.210	0.299
AK017277	*PTPRG*, Protein tyrosine phosphatase, receptor type G	Transmembrane receptor tyrosine kinase signaling pathway	0.230	0.331
NM_08045	*GJA12*, Gap junction membrane channel protein alpha 12	Cell-cell signaling	0.527	0.661
NM_08045	*GJE1*, Gap junction membrane channel protein epsilon 1	Cell-cell signaling	0.317	0.396
NM_03061	*AKR1C6*, Aldo-keto reductase family 1, member C6	Steroid biosynthesis	0.247	0.371
NM_03115	*APOBEC1*, Apolipoprotein B editing complex 1	Lipoprotein metabolism	0.459	0.462
NM_01004	*DGAT1*, Diacylglycerol O-transferase 1	2-acylglycerol O-acyltransferase activity	0.250	0.413
NM_00787	*DPAGT1*, Dolichyl-phosphate acetylglucosaminephosphotransferase 1	Dolichol-linked oligosaccharide biosynthesis	0.380	0.575
AK016135	*ETNK1*, Ethanolamine kinase 1	Phosphatidylethanolamine biosynthesis	0.552	0.660
NM_02133	*G6PC2*, Glucose-6-phosphatase, catalytic 2	Carbohydrate metabolism	0.519	0.568
NM_00808	*GAPDS*, Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	Carbohydrate metabolism	0.314	0.350
NM_01029	*GK2*, Glycerol kinase 2	Carbohydrate/glycerol metabolism	0.450	0.442
BC004801	*IDI1*, Isopentenyl-diphosphate delta isomerase	Cholesterol biosynthesis	0.373	0.384
NM_00941	*TPP2*, Tripeptidyl peptidase II	Proteolysis	0.576	0.591
NM_01888	*FMO2*, Flavin containing monooxygenase 2	Oxygen and reactive oxygen species metabolism	0.640	0.590
NM_01938	*CNTN6*, Contactin 6	Transport	0.285	0.304
AK012248	*XPO5*, Exportin 5	Protein binding, transport	0.373	0.437
AF141934	*SLC4A4*, Solute carrier family 4, member 4	Ion transport	0.267	0.388
NM_02371	*SLC01A6*, Solute carrier organic anion transporter family, member 1A6	Ion transport	0.193	0.293
AK014872	*SLC06D1*, Solute carrier organic anion transporter family, member 6D1	Ion transport	0.362	0.674
NM_01881	*NUP210*, Nucleoporin 210	Nuclear pore	0.417	0.465
AK021021	*CD47*, CD47 antigen	Positive regulation of phagocytosis	0.935	0.138
AK013765	*ECGF1*, Endothelial cell growth factor 1	Chemotaxis	0.316	0.417
AK004137	*RPP30*, Ribonuclease P/MRP 30 subunit	Protein biosynthesis	0.344	0.423
NM_0216	*NCSTN*, Nicastrin	Protein processing	0.529	0.576
AK020384	*ZFP142*, Zinc finger protein 142	Regulation of transcription	0.356	0.330
NM_01392	*ZFP354C*, Zinc finger protein 354C	Regulation of transcription	0.171	0.289
NM_02058	*ZFP467*, Zinc finger protein 467	Regulation of transcription	0.569	0.441
AK018054	*ZFP50*, Zinc finger protein 50	Protein binding	0.318	0.457
NM_00955	*ZFP40*, Zinc finger protein 40	DNA binding	0.268	0.326
NM_0084	*IFIH1*, Inter-alpha trypsin inhibitor, heavy chain 1	Copper ion binding	0.304	0.354
NM_0206	*JPH3*, Junctophilin 3	Locomotion	0.177	0.227
NM_01938	*CSPG2*, Chondroitin sulfate proteoglycan 2	Extracellular matrix	0.230	0.317
AK014599	*HYAL4*, Hyaluronoglucosaminidase 4	Unknown function	0.436	0.590

groups are shown in Table 1~2. Although at present it is unclear with what regulatory pathways these proteins are concerned, these findings will be useful for elucidation of nickel-induced toxicity. It was found that several genes involved in the ubiquitin system were differentially expressed: the genes coding for *UBE2I*, *CUL3* and *UBE2E1*. These genes are thought to play a role in the degradation of proteins unstable to be renatured. There

was increased expression of six genes (*NFKBA*, *TPT1*, *CD79A*, *CDC42*, *EDG6*, and *PDC*) that code for proteins related to signal transduction. These genes may be relevant to the regulation of the nickel-inducible genes identified in this study and/or other nickel-regulated genes. Nickel has been reported to affect DNA-protein interaction, which could alter transcriptional activity (Wang *et al.*, 1988). In this study, nickel inhibits the expression of several genes for DNA-protein binding proteins including the genes coding for *ZEP142, ZEP354C*, *ZEP40*, *ZEP467*, and *ZEP50*. Genes for 13 ribosomal proteins bundled tightly together, characterized by up-regulation >1.5-fold at 12 h nickel treatment, compared to the result at 6 h. There was altered expression of 10 genes for transport proteins. These genes were those coding for *SLC25A20*, *TXNDC4*, *COX7A2*, and *RAB2* that were up-regulated, and *XPO5*, *SLC4A4*, *SLCO1A6*, *SLCO6D1*, and *NUP210* that down-regulated. In addition, the down-regulation of genes that code for many metabolic enzymes was detected. These genes were those coding for *AKRIC6, APOBEC1*, *DGAT1*, *ETNK1*, *G6PC2*, *GAPDS*, and *GK2*. These changes may reflect the detrimental effects of nickel.

A functional categories of the genes expressed by nickel acetate in group 1 significantly different from the one in group 2. The expression of genes involved in protein synthesis, apoptosis, and cell growth was altered in group 1, whereas many metabolic enzymes, transporters, and binding proteins were altered in group 2 (Fig. 3).

RT-PCR was used to evaluate a subset of genes identified by microarray analysis as undergoing significant changes in expression. Specific primers were designed for selected genes and mRNA expression was checked by RT-PCR using β-actin as internal standard (Fig. 4). Densitometry analysis of PCR products confirmed down-regulation of *FAIM* genes and up-regulation of *GPX1* and *GADD45B*. The expression of these genes in RT-PCR were comparable to the results obtained by microarray.

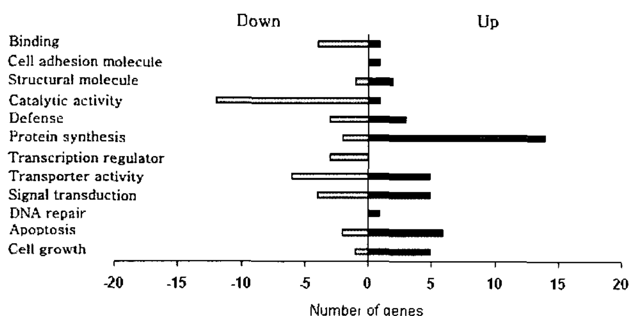

Fig. 3. Functional classification of 82 genes in response to nickel acetate. Bars indicate distribution of target genes showing the time-dependent changes in expression levels of differentially expressed genes in group 1 and 2.

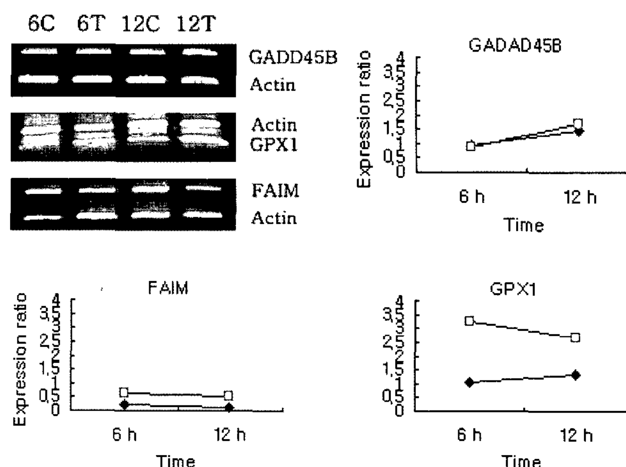

Fig. 4. Agarose gel electrophoretic pattern and comparison of expression for *FAIM, GPX1* and *GADD45B* genes in untreated and nickel acetate-treated mouse spleen cells for 12 h, as determined by microarray (◆) and quantitative RT-PCR (■). β-Actin primers (Ambion) were used to amplify as an internal standard. 6C, 6 h control; 6T, 6 h treated; 12C, 12 h control; 12T, 12 h treated. Expression ratio means the relative change in gene expression between control and nickel-treated cells.

DISCUSSION

This study was carried out to investigate the early gene response to nickel treatment using mice spleen cells. In our effort to identify nickel-responsive biomarkers and to understand the signal transduction pathways leading to cell cycle arrest and programmed cell death, we used the microarray technology to elucidate the changes in gene expression profile. Recently, microarray screenings of genes affected by nickel have been reported also by other researchers. They used different biological sources such a human peripheral lung epithelial cells or murine fibroblasts (Cheng *et al.*, 2003; Kowara *et al.*, 2005). Several of the genes detected in these studies were observed in common with our study. Although careful inspection is required before concluding the validity of altered expression, their data as well as ours might provide information about the tissue-specific effects of nickel. The spleen functions at several points in innate and adaptive immunity. The nature of changes in spleen cells exposed to nickel, which might be relevant to the postulated influence on immune system, was an open question. Because the spleen are rarely likely to be exposed to high concentrations, the changes at lower and nontoxic nickel concentration (40

μM) may be more relevant to the response of spleen in nickel toxicity. For this reason, we focused most attention on results in this range.

On the basis of the collection of 82 genes screened by two independent experiment, we have focused on genes associated with cell cycle controllers, apoptosis regulators, and DNA repair. Of these, four genes (*GADD45B, SOD1*, *GPX1*, and *FAIM*) were further characterized by RT-PCR, and the expression changes in three genes (*GADD45B*, *GPX1*, and *FAIM*) were verified. Because this is still an emerging technology and reliability of the microarray data has not been widely studied and published in the literature, there is little guidance to compare our experience with that of other investigators.

Genotoxic stress triggers a variety of cellular responses including the transcriptional activation of genes regulating DNA repair, cell cycle arrest, and apoptosis (Kwon *et al.*, 2005; Boiko *et al.*, 2006; Yasuda and Narumiya, 2006). Toxicity of nickel compounds is often associated with the induction of oxidative DNA damages, such as DNA strand breaks, DNA-DNA cross-links, DNA-protein cross-links, and aberrant DNA replication and repair. It was reported earlier that nickel inhibited repair of DNA strand breaks in cultured Chinese hamster ovary cells after UV-irradiation by inhibiting the ligation steps (Lee-Chen *et al.*, 1993). It is believed that oxidative DNA damage results from interaction of intracellular nickel with endogenous oxidants, which yield the DNA-damaging reactive oxygen species (ROS). Consistent with this, several genes that have anti-oxidative roles were up-regulated, including those coding for *MNSOD* (Mn superoxide dismutase) and *GPX1*. Inversely, increased expression of these genes may indicate that the nickel generates reactive oxygen in spleen cells. In addition, induction of DNA damage-response genes including *BTG2* and *GADD45B* may be a good indicator of DNA damage caused by nickel in mouse spleen cells. BTG2 regulates a wide variety of cell functions including transcriptional regulation and differentiation. BTG2 has also recently been shown to be crucial for negative regulation of cell proliferation by down-regulation of cyclin D1 and several studies pinpoint BTG2 as a tumor suppressor that links p53 and Rb pathways in human tumorigenesis (Kwon *et al.*, 2005; Boiko *et al.*, 2006). *GADD45* gene is known to be transcriptionally activated by p53-dependent or -independent pathway during the cellular response to DNA damage, which causes cell cycle arrest at the G2/M phase (Hirose *et al.*, 2003). Nickel-induced apoptosis and G2/M arrest have been reported by us and other researchers (Schedle *et al.*, 1995; Lee *et al.*, 1998; Shiao *et al.*, 1998). This implies that a molecular pathway involving GADD45 may contribute to the explanation of nickel-induced G2/M arrest.

On the basis of the results obtained from the present study, several targets or pathways that might be involved in the apoptotic response to nickel acetate were suggested on the array. Both proapototic and anti-apoptotic signals were altered in the spleens after nickel treatment. The induction of proapoptotic gene *GPX1* and decreased expression of anti-apoptotic gene *FAIM* were detected in the microarray and was validated by quantitative RT-PCR. Additionally, expression was increased for proapoptotic genes (*PDCD5* and *PLAGL2*) and anti-apoptotic genes (*MCL1*, *TPT1*, and *ANAPC11*). The main function of PDCD5 is believed to be the promotion of apoptosis by contributing to the enhancement of TAJ/TROY-induced parapoptotic cell death (Wang *et al.*, 2004). Thus, early response in spleen cells against to nickel treatment might produce a balance between apoptosis and anti-apoptotic signals. Even though some genes activate and other inhibit the function, a cell must maintain homeostasis and thus is likely to regulate any ongoing activity by balancing the expression of excitory and inhibitory factors. Also, this may be explained by the presence of mixed cell population at different stages of the cell cycle, which included the cells that were trying to induce apoptosis genes to eliminate the damaged cells, compared to the cell population that repaired the DNA damage and trying to slow down apoptosis by inducing anti-apoptotic genes. Further investigation is need to verify the possible functional roles of individual genes in the complex balance of cell survival and cell death. Some of the differentially expressed genes have previously been shown to regulate apoptosis and cell growth, whereas others have roles in the pathways that regulate cellular events such as protein synthesis, signaling, metabolism, cell adhesion, solute transport, cell growth, and defense, all of which are important in determining cell fate. Our studies suggest that the expression of many genes relevant to toxic effects by nickel and to protection from damage against it was modulated. For many of these changes in gene expression, biological significance of differentially expressed genes remains ambiguous. However, they are expected to serve as important clues to depict a general picture of nickel response.

In summary, our results indicate that nickel treatment on cultured mouse spleen cells led to moderate but significant changes in the expression of several genes, which might have important roles in the early stage of nickel-induced toxicity. The list of these genes could provide the basis of further systematic study of toxic or physiologic effects of nickel on spleen cell function.

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