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TOXICOGENOMICS

Gene expression profiling in the lung tissue of cynomolgus monkeys in response to repeated exposure to welding fumes

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Abstract Many in the welding industry suffer from bronchitis, lung function changes, metal fume fever, and diseases related to respiratory damage. These phenomena are associated with welding fumes; however, the mechanism behind these findings remains to be elucidated. In this study, the lungs of cynomolgus monkeys were exposed to MMA-SS welding fumes for 229 days and allowed to recover for 153 days. After the exposure and recovery period, gene expression profiles were investigated using the Affymetrix GeneChip® Human U133 plus 2.0. In total, it was confirmed that 1,116 genes were up-or down-regulated (over 2-fold changes, $P < 0.01$) for the T1 (31.4 ± 2.8 mg/m^3) and T2 (62.5 ± 2.7 mg/m^3) dose groups. Differentially expressed genes in the exposure and recovery groups were analyzed, based on hierarchical clustering, and were imported into Ingenuity Pathways Analysis to analyze the biological and toxicological functions. Functional analysis identified genes involved in immunological disease in both groups. Additionally, differentially expressed genes in common between monkeys and rats following welding fume exposure were compared using microarray data, and the gene expression of selected genes was verified by real-time PCR. Genes such as *CHI3L1*, *RARRES1*, and *CTSB* were up-regulated and genes such as *CYP26B1*, *ID4*, and *NRGN* were down-regulated in both monkeys and rats following welding fume exposure. This is the first comprehensive gene expression profiling conducted for welding fume exposure in monkeys, and these expressed genes are expected to be useful in helping to understand transcriptional changes in monkey lungs after welding fume exposure.

Keywords MMA-SS welding fumes · Monkey · Gene expression profiling

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Introduction

Welding fume exposure occurs in many industrial fields. It is estimated that approximately 800,000 full-time welders were exposed to welding fumes during welding. If welders working at part-time jobs are included, many more welders may be exposed worldwide (Sferlazza and Beckett 1991). Welding fumes are created when metal is united with pressure and heat. During this process, many injurious factors are generated, including welding fumes, ozone, and gases, such as nitric oxide and steam vapor, as well as ionizing and

non-ionizing radiation (Harris 2002; Burgess 1995). In particular, welding fume components, such as Fe, Cr, and Ni, can cause pulmonary disease (Antonini et al. 2004).

Many studies have been conducted regarding the injurious factors generated during the welding process. These studies have focused on the toxicological operation of the lungs during welding fume exposure. Furthermore, the correlation between the injurious components of welding fumes including pulmonary diseases, such as siderosis, immunosuppression, and lung cancer, has also been studied (Antonini et al. 2003). Acute exposure to welding fumes induces metal fume fever (Mueller and Seger 1985) and reversible respiratory symptoms (El-Zein et al. 2003; Wolf et al. 1997). Moreover, welding fumes induce asthma in welders, and there is an increase in the inflammatory transition of the lungs, such as in chronic bronchitis (El-Zein et al. 2003). These studies show that exposure to a high concentration of welding fumes over the long term induces pulmonary diseases. Yu et al. established that a stainless steel welding fume generation system produced pneumotoxic effects, and lung fibrosis was induced by exposure to chronic and high concentrations of welding fumes in Sprague–Dawley rats (Yu et al. 2001, 2003a, b, 2004). Although the toxicological effects of welding fumes on lung injury have been studied using animal models, information about the molecular and genetic events that cause lung injury or trigger the inflammatory response to prevent injury is lacking.

Recently, microarray analysis has been used in toxicology to interpret the toxicological effects at the transcriptional level and to identify genetic biomarkers in specific target cells or tissues (Young 2002, Chung et al. 2004; Oda et al. 2005; Powell et al. 2006). Moreover, phenotype-anchored gene expression profiles suggest that various toxicological endpoints or diseases can be classified or predicted by gene expression patterns (Alizadeh et al. 2000; Bittner et al. 2000). Gene expression analysis has been used to investigate peripheral blood mononuclear cells in which pneumoconiosis symptoms were caused in a rat model after a 30-day exposure to welding fumes (Rim et al. 2004). In a previous study, we also investigated gene expression profiling in lung injury in Sprague–Dawley rats after welding fume exposure and recovery (Oh et al. 2007). Although gene expression profiling has been performed in animal models, there are differences in transcriptomic regulation between humans and animals.

Thus, in this study, the cynomolgus monkey model, the genome of which is highly homologous to the human genome, was used to investigate gene expression profiling of lung injury following welding fume exposure. Gene expression profiling using a monkey model may reduce interspecies variances between an animal model and humans and help to address the toxicity of welding fume exposure in the human lung. We also compared gene expression profiles between the rat and the monkey to analyze the genetic level correlation and assess the reliability of expression patterns in the monkey model, because we used a limited number of monkeys in the study. This is the first comprehensive report on gene expression in the lungs of monkeys after welding fume exposure and recovery. This study provided molecular insights in the lung tissues when welding fumes were repeatedly infiltrated.

Materials and methods

Generation of MMA-SS welding fumes

The welding fumes were generated using an automatic robotic arm as a holding support for the welding rod (KST 308, 2.6 mm× 300 mm, Korea Welding Electrode Co. Ltd, Seoul, Korea) as previously described (Sung et al. 2007; Park et al. 2007). When the robotic arm approached the base stainless steel plate (SUS 304, 2.5 cm thick) in a zigzag motion, an arc was produced and the rod was consumed, generating welding fumes. The fumes were then moved into exposure chambers (whole-body type, each 1.5 m^3, Dusturbo, Seoul, Korea) that were rectangular in shape and made of metal with a Plexiglas window. Each chamber accommodated two monkey cages, and the total volume occupied by the two monkeys in a chamber was estimated as 1.3%. The chambers were equipped with HEPA filters to provide purified air to the exposure chambers. The welding fumes in the chamber were sampled using a personal sampler (MSA 484107, Pittsburgh, PA) at a flow rate of 2 l/min. The metal composition of the welding fume particulates captured on membrane filters (pore size 0.8 μm, 37 mm diameter, Millipore AAWP 03700, Bedford MA, USA) was analyzed for metal composition with an inductively coupled plasma analyzer (Thermojeralash, IRIS, Houston, TX, USA), using the NIOSH method 7300 (1999). Nitrous fumes, O_3, and NO_2 were all measured using Drager tubes (catalog numbers 6733181, CH 31001, and CH 30001, respectively) and sampled by stroking a gas detector pump (6400000, Drager, Lubeck, Germany), according to the manufacturer's directions 1 h after the welding fume exposure began. An Anderson sampler (AN-200, Shibata, Tokyo, Japan) was used to measure the mass media aerodynamic diameters of the welding fumes. The flow rate was 28.3 l/min, and the samples were collected for 5 min.

Exposure to welding fumes

Monkeys were exposed to the welding fumes as described previously (Sung et al. 2007; Park et al. (2007). Six

63 ± 5-month-old, male cynomolgus monkeys (3.7 ± 0.7 kg; *Macaca fascicularis*) were purchased from the Yunnan National Laboratory Primate Center (China) and acclimated for a 3-month period. The sequestered animal room was maintained at a temperature of 23 ± 3°C and a relative humidity of 55 ± 10%, with air ventilation 10–20 times/h, a light intensity of 150–300 lux, and a 12/12-h light/dark cycle (8 am to 8 pm). Throughout the study, the monkeys were individually housed in stainless steel wire cages (660 W × 800 l × 850 H mm) and fed a standard monkey diet (Oriental Yeast Co., Tokyo, Japan). No dietary supplement, such as fruit, was provided. Ultraviolet-irradiated and filtered municipal tap water was provided to the animals ad libitum. All animals were cared for in accordance with the principles outlined in the "Guide for the Care and Use of Laboratory Animals," an NRC publication (ILAR 1996). The monkeys were randomly assigned to three groups (unexposed, $n = 2$; low dose, $n = 2$; and high dose, $n = 2$), using the Path/Tox System (Version 4.2.2, Xybion Medical Systems Corporation, Cedar Knolls, NJ, USA), and exposed to welding fumes for 2 h/day, 5 days/week (1:30 pm to 3:30 pm) in the exposure chambers. Before initiating the inhalation exposure, the monkeys were taken out of their normal cages and housed in individual wire cages (450 W × 600 l × 460 H mm) that were specially designed for the inhalation experiment. In total, four monkeys, two in each chamber, were concurrently exposed during each 2-h exposure period. One monkey was used in each test group and recovery group. The control animals were not placed in the inhalation chamber; they remained in the cage during the 2-h exposure period. Food and water were not provided during the 2-h exposure, and the monkeys were taken out of the chambers at the end of the 2-h exposure. The time-weighted average (TWA) concentrations for the exposure doses were 31.4 ± 2.8 mg/m^3 (T1) and 62.5 ± 2.7 mg/m^3 (T2) total suspended particulates per 2 h. The target concentrations were achieved by varying the flow rates, by adjusting the dampers. Necropsies were performed after the 229 days of exposure and after the 153-day recovery period.

Histopathology

Lung samples collected from exposed, recovered, and control monkey were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (4 μm) were cut using a microtome (RM2165; Leica, Wetzlar, Germany), stained with hematoxylin and eosin, and examined under a light microscope (E400; Nikon, Tokyo, Japan).

Isolation of RNA

A portion of the lung samples was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the isolated total RNA was repurified using an RNeasy mini kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. Total RNA was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Montchanin, DE, USA), and the quality of RNA was evaluated using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) for DNA chip experiments.

Microarray analysis

The Affymetrix GeneChip® Human Genome U133 Plus 2.0 array was used for the microarray analysis. Sample labeling, microarray hybridization, washing, and scanning were performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). Microarray experiments for each exposure and recovery group were duplicated and, in total, twelve arrays were used. The preprocessing procedure for the cell intensity files (CEL) and the following microarray analyses were performed using GenPlex software (Istech Inc., Goyang, Korea). Data were normalized using global scale normalization. The differentially expressed genes in the each dose group of 229-day welding fume exposure group and the 153-day recovery group were selected based on the fold change and results from the Student's *t*-test (over 2-fold and $P < 0.01$), compared with the corresponding controls. Hierarchical clustering was also performed with the centered Pearson's correlation, using these selected genes, based on the complete linkage and distance matrix. Differentially expressed genes in the 229-day welding fume exposure group and the 153-day recovery group were imported into Ingenuity Pathways Analysis (IPA; Ingenuity Systems, Redwood, CA, USA), and the biological functions and toxicology were analyzed. Genes commonly deregulated during welding fume exposure between the monkeys and the rats were analyzed using microarray data for the 229-day welding fume exposure group of monkeys and those for the 30-day welding fume exposure group of rats, previously reported by Oh et al. (2007). In rat model for welding fume exposure, rats for T1 and T2 dose group were exposed to 51.4 ± 2.89 mg/m^3 and 84.63 ± 2.87 mg/m^3, respectively, for 2 h per day for up to 30 days (Oh et al. 2007). Lower cutoff threshold (over 1.3-fold and $P < 0.01$) for selecting the differentially expressed genes was performed to compare deregulated genes between two species exposed to welding fumes. Based on fold change and statistical significance, 1,342 and 4,881 differentially expressed genes were selected in the monkey and rat exposure groups, respectively. Among the 1,342 differentially expressed genes in monkeys, 534 genes with a gene symbol were selected to compare with those of the rat model. The selected genes were annotated based on NetAffx (http://www.Affymetrix.com).

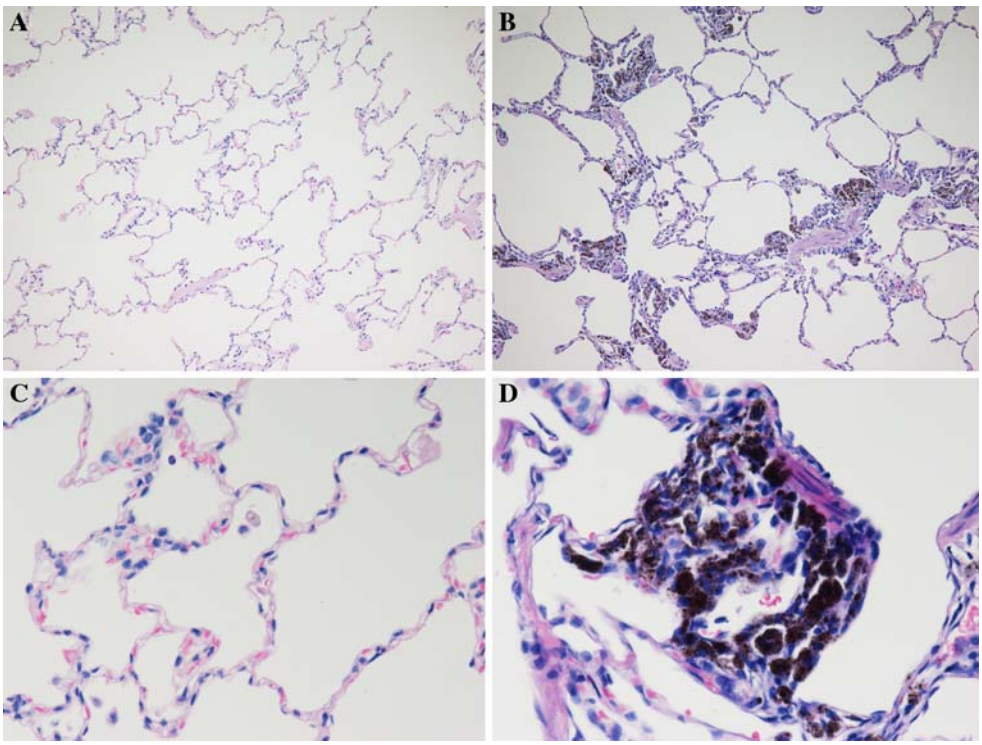

Fig. 1 Light micrographs of monkey lungs after 229 days of welding fume exposure **a** control (×100), **b** T2 dose (62.5 ± 2.7 mg/m^3, ×100), **c** Control (×400), **d** T2 dose (62.5 ± 2.7 mg/m^3, ×400)

Quantitative real-time RT-PCR

Gene transcripts were detected and quantified using SYBR Green (QuantiTect SYBR Green PCR Master Mix; Qiagen), according to the manufacturer's instructions, on a Rotor-Gene 6000 real-time rotary analyzer (Corbett Research, Sydney, Australia). Primers were designed using the Primer3 software (http://frodo.wi.mit.edu/); the primer sequences are presented in Supplemental Table 1. A melting curve analysis was performed on all amplified products to ensure the specificity and integrity of the PCR products. The *Gapdh* level was used as an internal control, and fold changes were calculated according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Results

Exposure to welding fumes and histopathology

To induce lung damage caused by welding fumes, monkeys were exposed to welding fumes at dose levels of 31.4 ± 2.8 mg/m^3 (T1 dose) and 62.5 ± 2.7 mg/m^3 (T2 dose) for 229 days and allowed to recover for 153 days. After the recovery period, serum biochemical and pathological examinations were performed. Serum biochemistry showed that no significant change was noticed (data not shown) in lymphocytes or neutrophils during the welding fume exposure. Histopathology showed that significant lung damage, such as pulmonary fibrosis, was not observed in either the 229-day exposure group or the 153-day recovery group. However, the lung tissues were infiltrated with welding fumes in both the T1 and T2 dose groups (Fig. 1). A similar severity of infiltration was interestingly observed in the 153-day recovery group (data not shown), even though after long-term recovery period (153-day).

Differentially expressed genes in the monkey lungs of the welding fume-exposed and recovery groups

For the microarray analysis, differentially expressed genes were selected from the monkey lung tissues in the welding fume exposure and recovery groups. In the exposure and recovery group, 669 (T1 dose, 365; T2 dose, 370) and 489 (T1 dose, 309; T2 dose, 239) genes were up- or down-regulated, respectively. Hierarchical clustering was performed; the results showed that samples were clustered in each dose group, many genes were commonly deregulated in both dose groups, and several genes were clustered specifically to each dose group (Fig. 2). The top 20 highly deregulated genes from the exposure group are shown in Table 1. Genes involved in signaling pathways (*DGKB*, *PIAS2*, *AXIN2*), metal ion binding (*TRIM2*), DNA binding (*HIST1*, *H2BC*), and metabolism (*CHIT1*) were up-

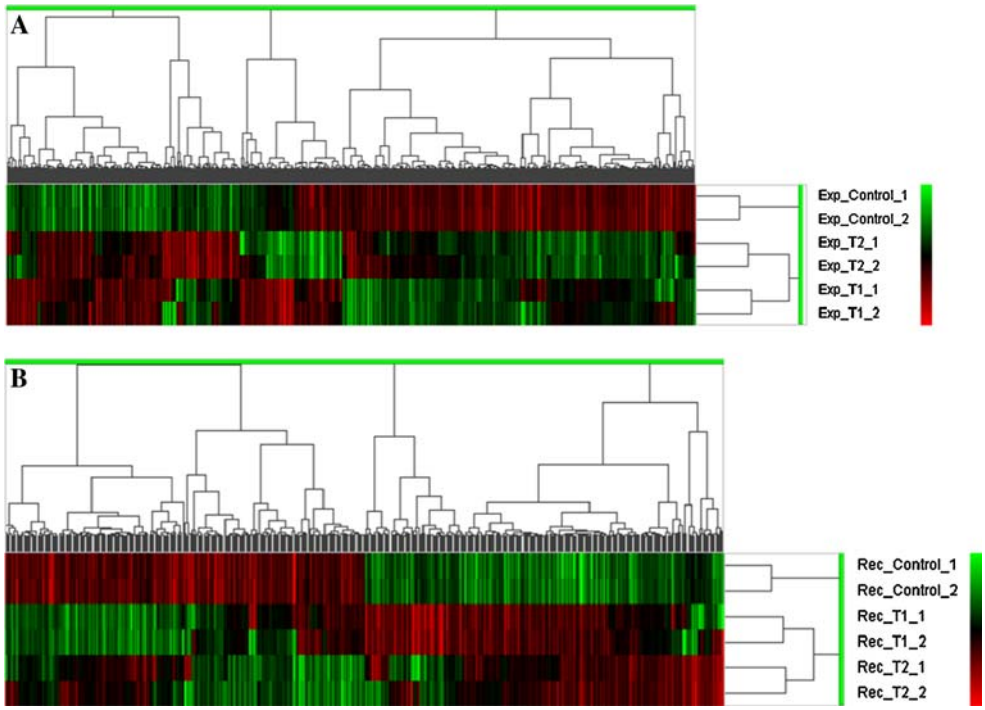

Fig. 2 Hierarchical clustering of differentially expressed genes in monkey lungs from the welding fume exposure **a** and recovery **b** groups

regulated in the exposure group, although most genes were not functionally annotated. In contrast, genes involved in transport (*ABCA13*, *STEAP2*, *KCNH2*, *KCNV1*), transcription (*236231_at*, *ZNF738*, *HEY2*), cell adhesion (*ACTN2*), rRNA processing (*ADAT2*), and protein binding (*SLITRK6*) were down-regulated in the exposure group.

In the recovery group, genes involved in tRNA aminoacylation (*IGL@, TARS*), antigen presentation or immune response (*HLA-DPB1, IGHM, GAGE12F*), cell differentiation or development (*THOC5, FNDC3A, DOCK7*), metabolism (*CHIT1, CPT1A*), and apoptosis (*240890_at*, *JAK2*) were up-regulated, whereas genes involved in heat shock protein binding (*DNAJC6*, *NTRK2*, *DNAJC10*), signal transduction (*RGS4*), proteolysis (*DPP10*), antigen presentation (*HLA-DPA1*), cell cycle arrest (*GAS2L3*), transcription (*ZNF483*), and development (*RICTOR*) were down-regulated (Table 2).

Functional classification of differentially expressed genes in the welding fume exposure and recovery groups

The molecular mechanisms of these selected 669 and 489 genes from the exposure and recovery groups, respectively, were analyzed using IPA. As shown in Table 3, the results confirmed changes in the expression of genes in the exposure group involved in immunological disease, genetic disorders, cancer, organism injury and abnormalities, and inflammatory diseases. In the recovery group, genes involved in cancer, immunological diseases, and inflammatory diseases ranked high. Among these categories, highly regulated genes related to immunological and inflammatory disease were represented in Table 4. As shown in Table 4, *PPID*, *CFLAR*, *CPT1A*, and *INSR* for up-regulated genes and *KLKB1*, *ATM*, *RAG1*, *UBASH3A*, *IGKC*, and *PTPN22* for down-regulated genes were consistently regulated in both exposure and recovery group.

When the molecular and cellular functions were analyzed, changes in the expression of genes involved in cellular growth, proliferation, and development were observed in the exposure group. Changes in the expression of genes involved in cellular growth, proliferation, and the cell cycle were also observed in the recovery group. In the analysis of toxicological functions, changes in genes involved in the G1/S transition of the cell cycle, TR/RXR activation, and hepatic fibrosis were identified in both the exposure and recovery groups. In particular, changes in genes involved in gene regulation mechanisms by peroxisome proliferation, RAR activation, and oxidative stress response mediated by Nrf2 were identified in the recovery group (Fig. 3).

Commonly deregulated genes in the lungs of monkeys and rats after welding fume exposure

To compare the results from the gene expression pattern in monkey lung tissues exposed to welding fumes with those seen in rats, the expression level of 534 genes with

Table 1 Differentially expressed genes in monkey lungs from welding fume exposure group

Gene_symbol /probe ID	Gene_title	RefSeq ID	Fold change (Log 2)	
			Exp_T1	Exp_T2
Up-regulated genes in the exposure group				
XIST	X (inactive)-specific transcript	NR_001564	3.28	8.43
TMED6	Transmembrane emp24 protein transport domain containing 6	NM_144676	3.65	5.10
SFRS4	Splicing factor, arginine/serine-rich 4	NM_005626	2.28	5.00
1556192_x_at	Full-length insert cDNA clone YR55D08	–	4.17	4.58
242830_at	Unknown	–	2.49	4.58
DGKB	Diacylglycerol kinase, beta 90 kDa	NM_004080	2.36	4.52
TRIM2	Tripartite motif-containing 2	NM_015271	0.38	4.48
EML5	Echinoderm microtubule-associated protein like 5	NM_183387	3.90	4.35
244388_at	Transcribed locus	–	3.24	4.06
C5orf28	Chromosome 5 open reading frame 28	NM_022483	4.74	4.05
1564299_at	CDNA FLJ33307 fis, clone BNGH42004076	–	2.91	4.01
1566836_at	CDNA clone IMAGE:5302735	–	3.53	3.89
HIST1H2BC	Histone cluster 1, H2bc	NM_003526	4.08	3.80
LOC339260	Hypothetical protein LOC339260	–	1.58	3.65
NHSL1	NHS-like 1	XM_496826	2.75	3.63
C6orf201	Chromosome 6 open reading frame 201	NM_001085401	3.25	3.61
PIAS2	Protein inhibitor of activated STAT, 2	NM_004671	1.17	3.58
AXIN2	Axin 2 (conductin, axil)	NM_004655	4.03	3.49
CHIT1	Chitinase 1 (chitotriosidase)	NM_003465	2.92	3.32
233010_at	CDNA FLJ14313 fis, clone PLACE3000341	–	3.67	3.30
Down-regulated genes in the exposure group				
OVOS2	Ovostatin 2	NM_001080502	−6.04	−5.85
ABCA13	ATP-binding cassette, sub-family A (ABC1), member 13	NM_152701	−3.64	−5.77
236945_at	Unknown	–	−1.89	−5.68
GPATCH2	G-patch domain containing 2	NM_018040	−0.20	−5.04
C20orf19	Chromosome 20 open reading frame 19	NM_018474	−0.30	−4.99
242818_x_at	Transcribed locus	–	−0.66	−4.95
TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2	NM_016192	−1.05	−4.91
KLKB1	Kallikrein B, plasma (Fletcher factor) 1	NM_000892	−0.32	−4.30
ACTN2	Actinin, alpha 2	NM_001103	−2.90	−4.03
236231_at	Unknown	–	−2.14	−4.01
1569772_x_at	CDNA clone IMAGE:4824424	–	−3.11	−3.98
ADAT2	Adenosine deaminase, tRNA-specific 2, TAD2 homolog (*S. cerevisiae*)	NM_182503	−2.37	−3.83
243548_x_at	Transcribed locus	–	−1.72	−3.75
ZNF738	Zinc finger protein 738	XR_015756	−2.76	−3.72
SLITRK6	SLIT and NTRK-like family, member 6	NM_032229	−1.83	−3.69
HEY2	Hairy/enhancer-of-split related with YRPW motif 2	NM_012259	−2.65	−3.66
STEAP2	Six transmembrane epithelial antigen of the prostate 2	NM_001040665	−0.74	−3.64
TEX12	Testis expressed 12	NM_031275	−2.40	−3.57
KCNH2	Potassium voltage-gated channel, subfamily H (eag-related), member 2	NM_000238	−1.92	−3.52
KCNV1	Potassium channel, subfamily V, member 1	NM_014379	−2.57	−3.48

Fold change was calculated with relative average value of 2 arrays in each group comparing to corresponding controls and values were represented with log 2

identical gene symbols were compared as described in the "Materials and methods" section. Of 534 monkey genes that showed changes in lung tissue, 76 matched changes in rats (15%). Among them, 39 were identified as up-regulated or down-regulated in both monkeys and rats (51%; Table 5). Most of these genes in common were down-

Table 2 Differentially expressed genes in monkey lungs from welding fume recovery group

Gene_symbol/probe ID	Gene_title	RefSeq ID	Fold change (Log 2)	
			Rec_T1	Rec_T2
Up-regulated genes in the recovery group				
IGL@	Immunoglobulin lambda locus	–	4.63	4.92
1557452_at	Full-length insert cDNA clone ZC19A03	–	5.15	4.64
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	NM_002121	4.53	4.60
THOC5	THO complex 5	NM_001002877	1.59	4.12
IGHM	Immunoglobulin heavy constant mu	–	2.66	3.94
C6orf12	Chromosome 6 open reading frame 12	XM_001132906	4.18	3.88
FNDC3A	Fibronectin type III domain containing 3A	NM_001079673	3.65	3.81
1561906_at	Homo sapiens, clone IMAGE:3626122	–	3.93	3.81
MRPL44	Mitochondrial ribosomal protein L44	NM_022915	1.99	3.75
CHIT1	Chitinase 1 (chitotriosidase)	NM_003465	2.45	3.73
DOCK7	Dedicator of cytokinesis 7	NM_033407	2.79	3.73
HOXA9	Homeobox A9	NM_152739	1.55	3.70
240890_at	CDNA clone IMAGE:5216666	–	2.56	3.65
CPT1A	Carnitine palmitoyltransferase 1A (liver)	NM_001031847	2.93	3.63
TARS	Threonyl-tRNA synthetase	NM_152295	4.09	3.56
1569727_at	Homo sapiens, similar to hypothetical gene LOC130797	–	3.12	3.51
GAGE12F	G antigen 6	NM_001098405	3.57	3.42
LOC731851	Hypothetical protein LOC731851	XM_001131041	3.69	3.37
JAK2	Janus kinase 2 (a protein tyrosine kinase)	NM_004972	3.40	3.35
C18orf17	Chromosome 18 open reading frame 17	NM_153211	1.27	3.33
Down-regulated genes in the recovery group				
RGS4	Regulator of G-protein signaling 4	NM_001102445	−3.25	−5.20
DPP10	Dipeptidyl-peptidase 10	NM_001004360	−1.42	−4.64
DNAJC6	DnaJ (Hsp40) homolog, subfamily C, member 6	NM_014787	−0.67	−4.61
NTRK2	Neurotrophic tyrosine kinase, receptor, type 2	NM_001007097	−0.85	−4.32
DNAJC10	DnaJ (Hsp40) homolog, subfamily C, member 10	NM_018981	−3.90	−4.03
HLA-DPA1	Major histocompatibility complex, class II, DP alpha 1	NM_033554	−1.64	−3.87
C11orf54	Chromosome 11 open reading frame 54	NM_014039	0.08	−3.85
GAS2L3	Growth arrest-specific 2 like 3	NM_174942	−2.55	−3.84
1560395_at	Homo sapiens, clone IMAGE:4293443, mRNA	–	−2.39	−3.81
FAM55C	Family with sequence similarity 55, member C	NM_145037	0.27	−3.62
229318_at	CDNA clone IMAGE:4814437	–	−2.92	−3.44
SPATA22	Spermatogenesis-associated 22	NM_032598	−2.50	−3.35
243302_at	Transcribed locus	–	−3.53	−3.33
1563397_at	EST from clone 114659, full insert	–	−1.86	−3.33
ZNF483	Zinc finger protein 483	NM_001007169	−1.42	−3.31
RICTOR	Rapamycin-insensitive companion of mTOR	NM_152756	−3.59	−3.29
244282_at	Transcribed locus	–	−1.25	−3.24
234650_at	CDNA: FLJ21254 fis, clone COL01317	–	−2.57	−3.23
240594_at	Transcribed locus	–	−3.30	−3.21
CYP26B1	Cytochrome P450, family 26, subfamily B, polypeptide 1	NM_019885	−3.06	−3.19

Fold change was calculated with relative average value of two arrays in each group comparing to corresponding controls, and values were represented with log 2

regulated. The common genes included *CHI3L1*, *GM2A*, *RARRES1*, *CTSK*, *DDHD1*, and *CTSB*. Among these, six genes that ranked high as either up-regulated or down-regulated genes were selected for real-time PCR to confirm gene expression (Fig. 4). Among the up-regulated lung genes from the monkey exposure group, *CHI3L1*,

Table 3 Functional classification of differentially expressed genes in the welding fume exposure or recovery group

Exposure			Recovery		
Functions	*P*-value	No. of genes	Functions	*P*-value	No. of genes
Disease and disorder					
Immunological disease	1.24E-05–1.32E-02	58	Cancer	1.55E-05–2.06E-02	109
Genetic disorder	2.19E-05–1.48E-02	152	Immunological disease	3.40E-05–2.06E-02	41
Cancer	5.55E-05–1.41E-02	148	Inflammatory disease	2.52E-04–2.06E-02	45
Organismal injury and abnormalities	5.65E-05–1.35E-02	27	Renal and urological disease	2.62E-04–2.06E-02	10
Inflammatory disease	1.06E-04–1.48E-02	59	Reproductive system disease	3.27E-04–2.06E-02	45
Molecular and cellular functions					
Cellular growth and proliferation	3.82E-08–1.35E-02	129	Cellular growth and proliferation	1.84E-06–2.06E-02	93
Cellular development	1.20E-06–1.35E-02	105	Cell cycle	7.30E-06–2.06E-02	38
Post-translational modification	8.86E-06–1.38E-02	43	Cell death	1.55E-05–2.06E-02	74
Cellular function and maintenance	1.16E-05–1.40E-02	22	Cell morphology	2.74E-05–2.06E-02	58
Cell cycle	2.22E-05–1.49E-02	52	Cellular development	1.56E-04–2.06E-02	67

Top functional categories for differentially expressed genes are presented for the exposure and recovery groups. *P*-values were calculated by comparing the number of molecules of interest relative to the total number of occurrences of these molecules in all functional annotations stored in the Ingenuity Pathways knowledge base (Fisher's exact test with *P*-value adjusted using the Benjamin–Hochberg multiple testing correction)

RARRES1, *DDHD1*, and *CTSB* were all up-regulated, but *GM2A* was down-regulated in rat lungs from the welding fume exposure group. However, selected down-regulated genes such as *GRAP*, *CYP1B1*, *PTGFRN ID4*, and *NRGN* in monkey lungs from the microarray analysis were all down-regulated in both monkey and rat samples. This overall result indicated that gene expression patterns detected from the microarray experiment were almost consistent with those determined from real-time PCR, and selected genes were consistently deregulated in rat samples.

Discussion

In this study, we analyzed the gene expression profiles from monkey lungs injured by welding fumes for 229 days and recovered for 153 days. Welding fumes consist of particulate matter from the heavy metal materials and gases, such as ozone. The Cr(VI) and nitrous fumes can include Fe, Mn, Ni, Cr, SiO_2, and asbestos (Antonini et al. 2004; Yu et al. 2001). Several studies have investigated the toxicological effects of welding fume exposure in various animal models (Hicks et al. 1983; Kalliomäki et al. 1986, Uemitsu et al. 1984). Gene expression changes should be triggered in target tissues by welding fume exposure, so microarray analysis is a useful tool for elucidating the molecular response to welding fume exposure. Rim et al. (2004, 2007) previously reported gene expression profiling of peripheral mononuclear cells from welding fume-exposed rats and welders. Gene expression profiling using blood samples could be useful to monitor the toxicological effects in surrogate tissues, but it is still of limited value in understanding dynamic phenomena, including lung inflammation or a response process in a target tissue. Actually, there were almost no genes consistently expressed in rat lungs (Oh et al. 2007) when compared with those expressed in rat blood after welding fume exposure (Rim et al. 2004). Furthermore, the use of a rodent model to predict toxic effects in humans also has limitations because of interspecies differences in toxicological responses, although central physiological functions are assumed to be almost common among mammals. For this reason, we used the monkey model to investigate gene expression profiling following welding fume exposure.

The histopathology showed that welding fumes were deposited within the lung tissues of monkeys, but there was no serious immune reaction. In a previous study, inflammation and infiltration of large numbers of immune cells into the alveoli were observed in a rat model following a 30-day welding fume exposure, and the lung almost recovered during a 30-day recovery period (Oh et al. 2007, 2009). These histological differences in welding fume exposure between monkeys and rats may have been caused by differences in breathing volumes of the animals, the respiration rate, and the actual exposed concentration of

Table 4 Top-regulated genes related to inflammation in monkey lungs

Gene_symbol	Gene_title	RefSeq ID	Fold change (Log 2)			
			Exp		Rec	
			T1	T2	T1	T2
Up-regulated genes						
PPID	Cyclophilin-40	NM_005038	0.59	2.62	−0.39	−1.45
INSR	Insulin receptor	NM_000208	1.90	2.55	−1.22	−0.94
CPT1A	Carnitine palmitoyltransferase 1A (liver)	NM_001031847	1.67	2.17	2.93	3.63
ALAS2	Aminolevulinate, delta-, synthase 2	NM_000032	0.53	1.63	−0.84	−0.26
CFLAR	I-FLICE isoform 5	NM_001127183	2.79	1.46	1.95	1.18
CDK2	Cyclin-dependent kinase 2	NM_001798	1.05	1.43	−1.17	−0.49
CPT1A	Carnitine palmitoyltransferase 1A (liver)	NM_001031847	0.83	1.39	0.64	0.74
F2RL1	Coagulation factor II (thrombin) receptor-like 1	NM_005242	0.26	1.18	−0.40	−0.06
PDE4D	Phosphodiesterase 4D, cAMP-specific	NM_001104631	−0.03	1.00	−0.50	−0.22
INSR	Insulin receptor	NM_000208	1.14	0.57	1.04	0.97
Down-regulated genes						
KLKB1	Kallikrein B, plasma (Fletcher factor) 1	NM_000892	−0.32	−4.30	0.08	−0.57
ATM	Ataxia telangiectasia mutated	NM_000051	−1.58	−3.24	−0.73	−0.53
RAG1	Recombination activating gene 1	NM_000448	−0.62	−3.20	−2.17	−2.51
UBASH3A	Ubiquitin associated and SH3 domain containing, A	NM_001001895	−1.05	−3.19	1.04	−0.45
IGKC	Immunoglobulin kappa constant	XM_001713938	0.22	−3.13	−1.44	−1.48
MAPK13	Mitogen-activated protein kinase 13	NM_002754	−1.62	−2.97	1.09	1.49
PTPN22	Protein tyrosine phosphatase, non-receptor type 22	NM_012411	−0.32	−2.93	−1.37	−0.39
MED7	Mediator complex subunit 7	NM_001100816	−1.52	−2.64	1.26	1.11
IGL@	Immunoglobulin lambda locus	–	2.88	−2.37	0.89	0.57
IFIH1	Interferon induced with helicase C domain 1	NM_022168	−1.48	−2.37	−0.58	−0.26

Fold change was calculated with a relative average value of two arrays in each group, compared with the corresponding controls
Values presented are log 2 transformed

welding fumes. In this study, exposure concentration of welding fumes was almost similar but the duration of welding fume exposure was different between monkey and rat models as follows: monkey model was exposed to 62.5 ± 2.7 mg/m^3 (T2 dose) for 229 days, and rats were exposed to 84.63 ± 2.87 mg/m^3 (T2 dose) for 30 days. Based on the respiratory rate between monkey (appx. 2,088 ml/min) and rat (appx. 264 ml/min) models, actual exposed concentration was estimated as previously described by Lawson (1998) and Authier et al. (2009). The actual exposed concentration was determined with 4.23 mg/kg/day and 9.68 mg/kg/day in monkey and rat models, respectively. Considering the duration of welding fume exposure, it was suggested that monkey was exposed to enough welding fumes, but welding fume accumulation in lungs has not been severe comparing to rat model. Moreover, in the monkey model, welding fumes were hardly removed from the lung after the 153-day recovery period. It seems that lung recovery or removal of welding fumes may be differently regulated in monkeys than rats. It was expected that we could understand and predict the molecular mechanism underlying welding fume exposure and the recovery process in humans using gene expression profiling in monkeys.

In the microarray analysis, the top-ranked differentially expressed genes involved in the inflammatory response were not primarily identified in the exposure and recovery groups of monkey lung, which differed from the many immune response genes identified in the rats investigated previously (Oh et al. 2007). However, a biofunctional analysis of all of the differentially expressed genes showed that about 50 genes identified in the exposure and recovery groups, respectively, appeared to be primarily involved in immunological disease. Table 4 represented that top-regulated genes related to inflammation in exposure or recovery group. Through analysis of expression changes for a total of 50 genes related to inflammation in exposure group comparing to recovery group, we found that about 50% of genes in T2 group were consistently up- or down-regulated in both exposure and recovery groups. This result

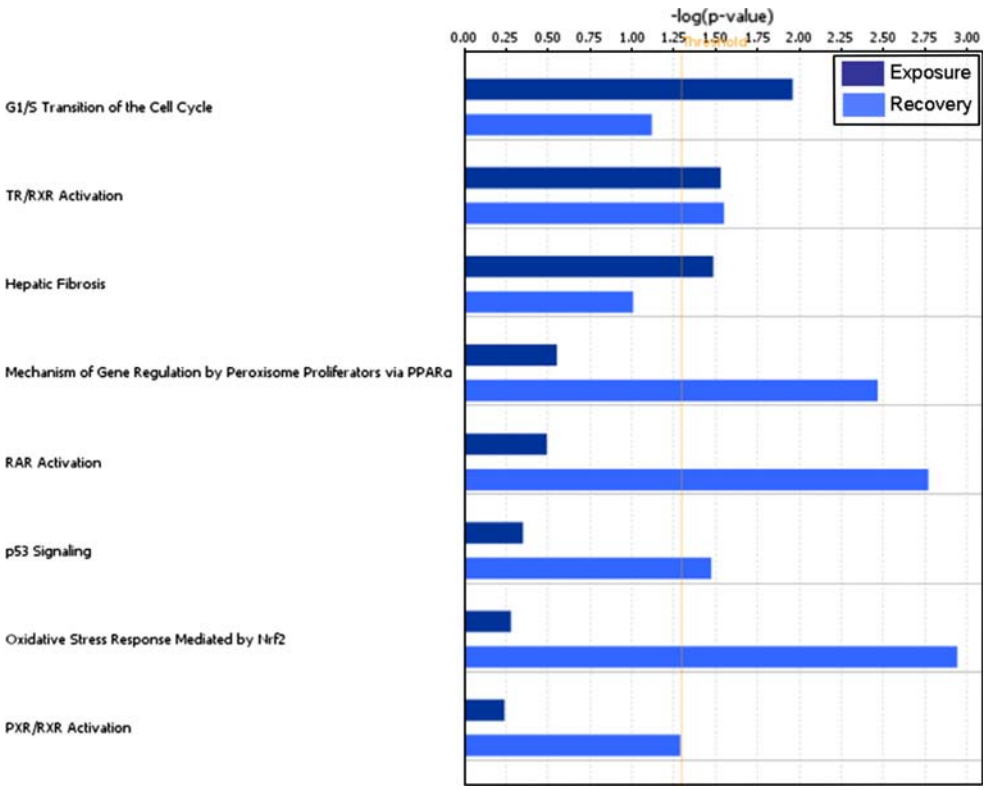

Fig. 3 Toxicological functional analysis of differentially expressed genes in the exposure and recovery groups. Interesting categories of mode of action were selected and represented. The *dark blue* and *light blue bars* in the histogram indicate the exposure and recovery groups, respectively

suggests that a significant inflammatory response did not occur in the lungs of welding fume-exposed monkeys but that inflammatory response was also progressed during recovery period.

Interestingly, there was a greater up-regulation of genes related to immunological disease in the recovery group than in the exposure group. The histopathology revealed that welding fumes were not removed during the 153-day recovery period, and it is thought that an inflammatory response increasingly progressed during the recovery period. Gene alterations involved in the immune response during the welding fume exposure and recovery periods were consistent with our histopathological observations. This result illustrates the utility of microarray analysis in characterizing responses to lung injury in monkeys exposed to welding fumes.

Here, we analyzed the changes in gene expression in the lungs of monkeys after welding fume exposure and recovery, but the number of individuals in each group was small. For this reason, we compared the differentially expressed genes identified in the present study with those identified in welding fume-exposed rats, which were previously reported (Oh et al. 2007). Among the commonly deregulated genes in the monkey and rat after welding fume exposure, *CHI3L1*, *CTSK*, and *CTSB* were up-regulated, whereas *GRAP*, *CYP1B1*, *CYP26B1*, and *ID4* were down-regulated, and the transcriptional alterations were also confirmed by real-time PCR. Transcriptional expression of *CHI3L1* is regulated by *TNF* or *IL1B* and *CHI3L1*, which are involved in macrophage differentiation (Recklies et al. 2005; Rehli et al. 2003). *CHI3L1* may play an important role in the early immune response in both monkeys and rats after exposure to welding fumes. Cathepsin K (*CTSK*), which is expressed in breast cancers, is also involved in the dendritic cell or macrophage signaling pathway and is also associated with differentiation in a leukemia cell line (Takeshita and Ishii 2008; Hattori et al. 2007). Additionally, cathepsin B (*CTSB*), which was up-regulated during welding fume exposure, is associated with apoptosis and proliferation in various cell lines, including lung cancer and fibroblast cell lines (Moubarak et al. 2007; Bröker et al. 2004). *GRAP*, which was down-regulated during welding fume exposure, plays a role in negatively regulating the proliferation of lymphocyte interleukin-2 induction (Shen et al. 2002). *CYP1B1* and *CYP26B1* were highly down-regulated during welding fume exposure.

To date, studies about xenobiotic metabolism induced by welding fume exposure are limited, and the mechanisms

Table 5 Commonly deregulated genes in the monkey and rat welding fume exposure groups

Gene_symbol	Gene_title	RefSeq ID		Fold change (Log 2)			
		Monkey	Rat	Monkey		Rat[a]	
				T1	T2	T1	T2
Up-regulated genes							
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	NM_001276	NM_053560	4.24	2.67	1.02	1.44
GM2A	GM2 ganglioside activator	NM_000405	NM_172335	1.12	1.55	0.59	0.58
RARRES1	Retinoic acid receptor responder (tazarotene induced) 1	NM_002888	NM_001014790	1.81	1.53	1.50	2.10
CTSK	Cathepsin K	NM_000396	NM_031560	1.28	1.41	1.37	1.37
DDHD1	DDHD domain containing 1	NM_030637	NM_001033066	1.61	0.96	0.52	0.68
CTSB	Cathepsin B	NM_001908	NM_022597	1.03	0.77	0.57	0.86
Down-regulated genes							
GRAP	GRB2-related adaptor protein	NM_006613	NM_001025749	−2.10	−2.69	−0.76	−0.54
CYP1B1		NM_000104	NM_012940	−2.71	−2.60	−0.53	−1.00
CYP26B1	Cytochrome P450, family 26, subfamily B, polypeptide 1	NM_019885	NM_181087	−1.23	−1.95	−0.66	−0.07
PTGFRN	Prostaglandin F2 receptor negative regulator	NM_020440	NM_019243	−1.63	−1.91	−0.59	−0.55
ID4	Inhibitor of DNA-binding 4, dominant negative helix-loop-helix protein	NM_001546	NM_175582	−1.35	−1.65	−1.82	−0.97
NRGN	Neurogranin (protein kinase C substrate, RC3)	NM_006176	NM_024140	−2.51	−1.60	−0.71	−0.43
KIDINS220	Kinase D-interacting substrate of 220 kDa	NM_020738	NM_053795	−1.08	−1.55	−0.38	−0.49
ANK2	Ankyrin 2, neuronal	NM_001148	XM_001076082	−2.15	−1.40	−1.01	−1.16
TMPO	Thymopoietin	NM_001032283	NM_012887	−1.31	−1.30	−0.39	−0.27
PTGER4	Prostaglandin E receptor 4 (subtype EP4)	NM_000958	NM_032076	−1.42	−1.27	−0.44	−0.47
RHOJ	Ras homolog gene family, member J	NM_020663	NM_001008320	−1.47	−1.27	−0.68	−0.62
CXCL12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	NM_000609	NM_001033882	−1.96	−1.25	−0.59	−0.18
RBP1	Retinol-binding protein 1, cellular	NM_002899	NM_012733	−1.26	−1.21	−0.92	−0.84
MAMDC2	MAM domain containing 2	NM_153267	XM_001078660	−1.59	−1.19	−0.84	−0.48
SPON1	Spondin 1, extracellular matrix protein	NM_006108	NM_172067	−1.40	−1.05	−0.50	−0.48
GHR	Growth hormone receptor	NM_000163	NM_017094	−1.20	−1.02	−0.86	−1.07
FXYD1	FXYD domain containing ion transport regulator 1 (phospholemman)	NM_005031	NM_031648	−1.26	−0.96	−0.93	−0.87
HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)	NM_000860	NM_024390	−1.31	−0.92	−1.05	−1.11
NBL1	Neuroblastoma, suppression of tumorigenicity 1	NM_005380	NM_031609	−1.04	−0.91	−0.68	−0.58
WNT5A	Wingless-type MMTV integration site family, member 5A	NM_003392	NM_022631	−1.14	−0.91	−0.72	−1.02
FHL1	Four and a half LIM domains 1	NM_001449	NM_001033926	−1.11	−0.87	−0.58	−0.56
KCNS3	Potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	NM_002252	NM_031778	−1.12	−0.73	−0.84	−0.56
SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporters), member 2	NM_001046	NM_031798	−1.19	−0.69	−0.45	−0.35
ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	NM_002221	NM_019312	−1.09	−0.63	−0.86	−0.81
SOX9	SRY (sex-determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	NM_000346	XM_001081628	−1.07	−0.62	−1.34	−1.18
G0S2	G0/G1switch 2	NM_015714	NM_001009632	−1.03	−0.60	−1.02	−0.77
IGFBP6	Insulin-like growth factor binding protein 6	NM_002178	NM_013104	−1.04	−0.55	−0.33	−1.04
RAB28	RAB28, member RAS oncogene family	NM_001017979	NM_053978	−1.12	−0.51	−0.38	−0.36
GBA2	Glucosidase, beta (bile acid) 2	NM_020944	NM_020944	−1.18	−0.51	−0.49	−0.32
HEY1	Hairy/enhancer-of-split related with YRPW motif 1	NM_001040708	XM_001057389	−1.05	−0.46	−0.50	−0.63
PDLIM3	PDZ and LIM domain 3	NM_014476	NM_053650	−1.61	−0.41	−0.76	−0.80
HNRPD	Heterogeneous nuclear ribonucleoprotein D)	NM_001003810	NM_001082539	−2.76	−0.30	−0.59	−0.39
RHOB	Ras homolog gene family, member B	NM_004040	NM_022542	−1.39	−0.12	−0.80	−0.61

Fold change was calculated with a relative average value of two (monkey model) or three arrays (rat model) in each group, compared with the corresponding controls. Values presented are log 2 transformed

[a] Microarray data in the rat model were used with the permission of Oh et al. (2007). Differentially expressed genes were compared as described in the "Materials and methods". T1 dose means 31.4 ± 2.8 mg/m^3 51.4 ± 2.89 mg/m^3 and T2 dose means 62.5 ± 2.7 and 84.63 ± 2.87 mg/m^3 in monkey and rat models, respectively

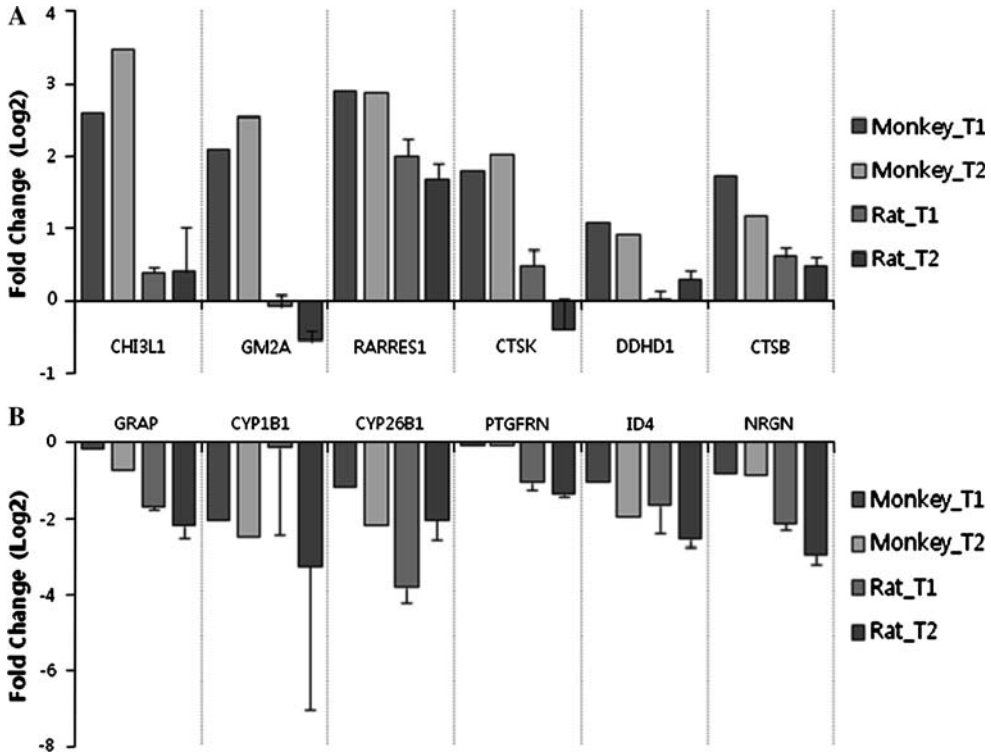

Fig. 4 Verification of top-ranked genes deregulated in monkey lung after welding fume exposure. Expression patterns of selected genes detected from the microarray experiment for monkey lung were analyzed in both the T1- and T2-dosed monkey and rat lungs by real-time PCR. **a** Six up-regulated genes, **b** Six down-regulated genes in the welding fume exposure group of monkeys. Three independent rat samples were used to confirm the gene expression levels and average fold change. The standard deviation was calculated as described in the "Materials and methods" section

are poorly understood. However, we found that *CYP1B1* and *CYP26B1* were deregulated in the lung after welding fume exposure. In contrast, *ID4*, a transcriptional regulator and inhibitor of DNA binding, was down-regulated during welding fume exposure. *ID4* plays an important role in the differentiation and proliferation of neural cell and epithelial cell lines (Shan et al. 2003; Yun et al. 2004), but its involvement in lung injury and lung inflammation has not been reported.

In a previous study, genes related to the immune response, such as *Mmp12* and *Trem2*, and many cytokines, such as *Cd5l*, *Ccl7*, and *Cxcl5*, were highly expressed in rats after welding fume exposure (Oh et al. 2007). In the present study, *MMP12* was not differentially expressed in monkey lung, but *MMP9* was up-regulated, while its expression was not altered in rats. *TREM2* was consistently up-regulated in both monkeys and rats, but *TREM2* was excluded from the gene list, because its gene symbol did not match during the analysis. A previous study showed that *MMP12* was sensitively and significantly up-regulated by welding fume exposure. This difference in gene expression might be due to the degree of lung injury induced by welding fumes or to interspecies variability. This result also suggests that *TREM2* plays an important role in lung injury induced by welding fume exposure in both monkeys and rats. In the case of cytokine genes, *CD5L*, *CCL7*, and *CXCL5* were up-regulated over 1.3-fold or 2-fold in the recovery group but not in the exposure group, although *P*-value was not over 0.01. Gene expression changes of these cytokines also indicate that lung injury was chronically progressed even through recovery period.

Using microarray analysis, we demonstrated, for the first time, a comprehensive gene expression profile in monkeys after welding fume exposure and recovery. We identified several genes commonly deregulated that are involved in inflammatory response and proliferation in both monkeys and rats after welding fume exposure. This information could aid in understanding the mechanisms in lung tissues after welding fume exposure.

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References

Alizadeh AA, Eisen MB, Davis R et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503–511

Antonini JM, Taylor MD, Zimmer AT et al (2003) Pulmonary responses to welding fumes: role of metal constituents welding. J Toxicol Environ Health A 67:233–249

Antonini JM, Taylor MD, Millecchia L et al (2004) Suppression in lung defenses after bacterial infection in rats pretreated with different welding fumes. Toxicol Appl Pharmacol 200:206–218

Authier S, Legaspi M, Gauvin D et al (2009) Respiratory safety pharmacology: positive control drug responses in Sprague-Dawley rats, Beagle dogs and cynomolgus monkeys. Regul Toxicol Pharmacol 55:229–235

Bittner M, Meltzer P, Chen Y et al (2000) Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 406:536–540

Bröker LE, Huisman C, Span SW et al (2004) Cathepsin B mediates caspase-independent cell death induced by microtubule stabilizing agents in non-small cell lung cancer cells. Cancer Res 64:27–30

Burgess WA (1995) Recognition of health hazards in industry-A review of materials and processes, 2nd edn. Wiley, New York, pp 169–179

Chung H, Hong DP, Jung JK et al (2004) Comprehensive analysis of differential gene expression profiles on carbon tetrachloride induced rat liver injury and regeneration. Toxicol Appl Pharmacol 206:27–42

El-Zein M, Malo JL, Infante-Rivard C et al (2003) Prevalence and association of welding related systemic and respiratory symptoms in welders. Occup Environ Med 60:655–661

Harris MK (2002) Welding health & safety: a field guide for OEHS professionals. American Industrial Hygiene Association (AIHA). Fairfax, Virginia

Hattori H, Zhang X, Jia Y et al (2007) RNAi screen identifies UBE2D3 as a mediator of all-trans retinoic acid-induced cell growth arrest in human acute promyelocytic NB4 cells. Blood 110:640–650

Hicks R, Al-Shamma KJ, Lam HF et al (1983) An investigation of fibrogenic and other toxic effects of arc-welding fume particles deposited in the rat lung. J Appl Toxicol 3:297–306

ILAR (Institute for Laboratory Animal Research) (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington, DC

Kalliomäki PL, Hyvärinen HK, Aitio A et al (1986) Kinetics of the metal components of intratracheally instilled stainless steel welding fume suspensions in rats. Br J Ind Med 43:112–119

Lawson PT (1998) Assistant laboratory animal technician training manual. American Association for Laboratory Animal Science, Memphis

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). Methods 25:402–408

Moubarak RS, Yuste VJ, Artus C et al (2007) Sequential activation of poly(ADP-ribose) polymerase 1, calpains, and Bax is essential in apoptosis-inducing factor-mediated programmed necrosis. Mol Cell Biol 27:4844–4862

Mueller EJ, Seger DL (1985) Metal fume fever-a review. Am J Emerg Med 2:271–274

NIOSH (1999) NIOSH manual of analytical methods. Method No. 7300, 7604. National Institute for Occupational Health, Cincinnati, OH

Oda H, Fushimi F, Kato M et al (2005) Microarray analysis of the genes induced by tetracycline-regulated expression of NDRF/NeuroD2 in P19 cells. Biochem Biophys Res Commun 335(2):458–468

Oh JH, Park HJ, Heo SH et al (2007) Gene expression analysis of lung injury in rats induced by exposure to MMA-SS welding fume for 30 days. Mol Cell Toxicol 3:306–313

Oh JH, Yang MJ, Yang YS et al (2009) Microarray-based analysis of the lung recovery process after stainless-steel welding fume exposure in Sprague-Dawley rats. Inhal Toxicol 21:347–373

Park JD, Chung YH, Kim CY et al (2007) Comparison of high MRI T1 signals with Mn concentration in brains of cynomolgus monkeys after 8 mo of stainless steel welding-fume exposure. Inhal Toxicol 19:965–971

Powell CL, Kosyk O, Ross PK et al (2006) Phenotypic anchoring of acetaminophen-induced oxidative stress with gene expression profiles in rat liver. Toxicol Sci 93:213–222

Recklies AD, Ling H, White C et al (2005) Inflammatory cytokines induce production of CHI3L1 by articular chondrocytes. J Biol Chem 280:41213–41221

Rehli M, Niller HH, Ammon C et al (2003) Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. J Biol Chem 278:44058–44067

Rim KT, Park KK, Sung JH et al (2004) Gene-expression profiling using suppression subtractive hybridization and cDNA microarray in rat mononuclear cells in response to welding-fume exposure. Toxicol Ind Health 20:77–88

Rim KT, Park KK, Kim YH et al (2007) Gene-expression profiling of human mononuclear cells from welders using cDNA microarray. J Toxicol Environ Health A 70:1264–1277

Sferlazza SJ, Beckett WS (1991) The respiratory health of welders. Am Rev Respir Dis 143:1134–1148

Shan L, Yu M, Qiu C et al (2003) Id4 regulates mammary epithelial cell growth and differentiation and is over expressed in rat mammary gland carcinomas. Am J Pathol 163:2495–2502

Shen R, Ouyang YB, Qu CK et al (2002) Grap negatively regulates T-cell receptor-elicited lymphocyte proliferation and interleukin-2 induction. Mol Cell Biol 22:3230–3236

Sung JH, Kim CY, Yang SO et al (2007) Changes in 470 blood manganese concentration and MRI T1 relaxation time during 180 days of stainless steel welding-fume exposure in cynomolgus monkeys. Inhal Toxicol 19:47–55

Takeshita F, Ishii KJ (2008) Intracellular DNA sensors in immunity. Curr Opin Immunol 20:383–388

Uemitsu N, Shimizu Y, Hosokawa T et al (1984) Inhalation toxicity study of welding fumes: effect of single or repeated exposure on the lungs of rats. Toxicology 30:75–92

Wolf C, Pirich C, Valic E et al (1997) Pulmonary function and symptoms of welders. Int Arch Occup Environ Health 69:350–353

Young RR (2002) Genetic toxicology: web resources. Toxicology 173:103–121

Yu IJ, Song KS, Chang HK et al (2001) Lung fibrosis in Sprague-Dawley rats, induced by exposure to manual metal arc-stainless steel welding fumes. Toxicol Sci 63:99–106

Yu IJ, Song KS, Chang HK et al (2003a) Recovery from manual arcstainless steel welding-fume exposure induced lung fibrosis in Sprague-Dawley rats. Toxicol Lett 143:247–259

Yu IJ, Park JD, Park ES et al (2003b) Manganese distribution in brains of Sprague Dawley rats after 60 days of stainless steel welding fume exposure. Neurotoxicology 24:777–785

Yu IJ, Song KS, Maeng SH et al (2004) Inflammatory and genotoxic responses during 30 day welding-fume exposure period. Toxicol Lett 154:105–115

Yun K, Mantani A, Garel S et al (2004) Id4 regulates neural progenitor proliferation and differentiation in vivo. Development 131:5441–5448