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Gene expression profile in circulating mononuclear cells after exposure to ultrafine carbon particles

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Abstract

Context—Exposure to particulate matter (PM) is associated with systemic health effects, but the cellular and molecular mechanisms are unclear.

Objective—We hypothesized that, if circulating mononuclear cells play an important role in mediating systemic effects of PM, they would show gene expression changes following exposure.

Materials and methods—Peripheral blood samples were collected before (0 hour) and at 24 hours after exposure from healthy subjects who participated in previous controlled exposures to ultrafine carbon particles (UFP, 50 $\mu\text{g}/\text{m}^3$) or filtered air (FA)(n = 3 each). RNA from mononuclear cell fraction (>85% lymphocytes) was extracted, amplified and hybridized to Affymetrix HU133 plus 2 microarrays.

Results—We identified 1713 genes (UFP 24 hours vs. FA 0 and 24 hours, $p < 0.05$, FDR 0.01). The top 10 upregulated genes (fold) were *CDKN1C* (1.86), *ZNF12* (1.83), *SRGAP2* (1.82), *FYB* (1.79), *LSM14B* (1.79), *CD93* (1.76), *NCSTN* (1.70), *DUSP6* (1.69), *TAC1* (1.68) and *H2AFY* (1.68). Upregulation of *CDKN1C* and *SRGAP2* was confirmed by RT-PCR using samples from additional 5 subjects exposed to FA and UFP. We entered 1020 genes with a ratio >1.1 or <-1.1 into the Ingenuity Pathway Analysis and identified many canonical pathways related to inflammation, tissue growth and host defense against environmental insults, including IGF-1 signaling, insulin receptor signaling and NRF2-mediated oxidative stress response pathway.

Discussion and conclusions—Two-hour exposures to UFP produced gene expression changes in circulating mononuclear cells. These gene changes provide biologically plausible links to PM-induced systemic health effects, especially those in the cardiovascular system and glucose metabolism.

INTRODUCTION

Short- and long-term exposures to ambient particulate matter (PM) are associated with a host of adverse systemic health effects, including myocardial ischemia and infarction, heart failure, arrhythmias, strokes and increased cardiovascular mortality (Brook, 2008). The mechanisms by which PM inhaled into the lung may instigate remote systemic health effects

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DECLARATION OF INTEREST

The authors report no declarations of interest. System and glucose metabolism

are intriguing. Evidence from acute in vitro toxicological experiments, controlled animal and human exposures and human panel studies have indicated three general pathways: systemic propagation of pulmonary inflammation and/or oxidative stress signals, alterations in the balance of autonomic nervous system, and direct effects on the vasculature by particles or particle constituents capable of reaching the systemic circulation.

The ultrafine fraction of ambient PM (diameter < 100 nm) has been proposed as a major contributor to the pulmonary and systemic adverse effects of ambient pollutant particles (Oberdorster and Utell, 2002, Seaton et al., 1995, Oberdorster et al., 1995). Although contributing little to the PM mass, the ultrafine fraction of PM has a higher number concentration and surface area compared with other fractions at similar mass concentrations. The large surface area may enhance oxidative stress and inflammation in the lung after inhalation. The biological signals generated by lung cells may then be transmitted by circulating cells to systemic organs. The ultrafine particles are deposited in more distal lung regions (Anderson et al., 1990), and may reach the vascular space and systemic organs more easily than fine or coarse fractions via some undefined transport mechanisms (Oberdorster et al., 2002, Nemmar et al., 2001, Nemmar et al., 2002). Despite the greater potential of ultrafine particles to produce biological effects, relatively few studies have directly assessed the effects of ambient ultrafine particles on health outcomes (Ibald-Mulli et al., 2002). The number of ultrafine particles was associated with reduced peak expiratory flow and increased respiratory symptoms in asthmatics (Peters et al., 1997, Penttinen et al., 2001). Exposure to ultrafine particles may be associated with increased mortality from respiratory and cardiovascular causes (Wichmann et al., 2000).

Previous human controlled exposure studies have shown that a 2-h exposure to ultrafine carbon particles produced pulmonary and systemic vascular effects (Frampton, 2001, Frampton et al., 2004, Pietropaoli et al., 2004, Shah et al., 2008). In addition, exposure to these carbon particles also altered peripheral blood leukocyte distribution and expression of adhesion molecules (Frampton et al., 2006). Although the mechanisms for the systemic effects of ultrafine carbon particles are unclear, the circulating leukocyte may be a candidate cell that carries biological signals from the lung to the systemic organs. In this study, we hypothesized that, if circulating mononuclear cells play an important role in mediating systemic effects of PM, they would show gene expression changes following exposure to ultrafine particles.

MATERIALS AND METHODS

Subjects

The study was approved by the Research Subjects Review Board at the University of Rochester, and subjects provided written informed consent before the controlled exposures and collection of blood samples. Subjects were healthy non-smokers (18–40 years of age), required to have no history of pulmonary or cardiovascular disease, normal spirometry, a normal electrocardiogram, and a negative urine pregnancy test (females). Subjects were instructed to exclude anti-inflammatory drugs for the duration of the study and to exclude caffeine, large fatty meals, and vigorous exercise during and 24 hr before study days. Subjects were not studied within 6 weeks of a respiratory infection. The subjects in this study all participated in a previous controlled exposure study to ultrafine carbon particles (UFP) (see below) (Frampton et al., 2006, Pietropaoli et al., 2004). Blood samples used in this study were collected during the exposure sessions.

UFP exposure

The studies used a double blinded, randomized (blocked by order of presentation and sex), crossover design. Exposures were undertaken within an environmental chamber, using a mouthpiece exposure system. Subjects underwent a 2-hr mouthpiece exposure to either filtered air (FA) or UFP ($50 \mu\text{g}/\text{m}^3$), with four 15-min exercise periods on a bicycle ergometer (target minute ventilation $20 \text{ L}/\text{min}/\text{m}^2$ body surface area). A 10-min break off the mouthpiece was taken after 1 hr of exposure. The environmental chamber was a one-pass, dynamic-flow exposure system. The particles (median aerodynamic diameter $\sim 25 \text{ nm}$; geometric standard deviation ~ 1.6) were generated in an argon atmosphere using an electric spark discharge between two graphite electrodes in a commercial generator (Palas Co., Karlsruhe, Germany) that had been modified to prevent any offgassing of organic materials from within the generator (McDonald et al. 2001). This produced particles consisting of $> 95\%$ elemental carbon without metals. The particles were then deionized and diluted with filtered air to the desired concentration. Particles were continuously generated, and the exposure concentration was monitored and regulated during the exposure. Particle number, mass, and size distributions were monitored on both the inspiratory and expiratory sides of the subject. The subject inhaled from a mouthpiece and wore a nose clip. One-way valves (Hans-Rudolph, Inc., Kansas City, MO) prevented rebreathing of UFP. Air for the control exposures and for dilution of the particles was passed through charcoal and high-efficiency particle filters and was essentially free of particles ($0\text{--}10 \text{ particles}/\text{cm}^3$).

Collection of circulating mononuclear cells

Peripheral blood samples were collected before (0 hour) and 24 hours after exposure ($n = 3$ FA and 3 UFP). The mononuclear cell fraction was isolated using BD Vacutainer™ CPT™ Citrate Tube (Becton-Dickinson, Inc., Franklin Lakes, NJ). The mononuclear cell fraction contains $> 85\%$ lymphocytes.

RNA extraction and hybridization

RNAs were extracted using TRIZOL®LS (Invitrogen Corp., Carlsbad, CA), amplified by the Affymetrix Two-Cycle cDNA synthesis kit and hybridized to Affymetrix HU133 plus 2 microarrays containing 54,675 probes (Affymetrix, Inc., Santa Clara, CA). The hybridization was performed according to the “Affymetrix Technical Manual”. Briefly, total RNA ($10 \mu\text{g}$) was converted into cDNA using Reverse Transcriptase (Invitrogen Corp., Carlsbad, CA) and a modified oligo(dT)24 primer that contains T7 promoter sequences (GenSet Corp., San Diego, CA). After first strand synthesis, residual RNA was degraded by the addition of RNaseH and a double-stranded cDNA molecule was generated using DNA polymerase I and DNA ligase. The cDNA was then purified and concentrated using a phenol:chloroform extraction followed by ethanol precipitation. The cDNA products were incubated with T7 RNA Polymerase and biotinylated ribonucleotides using an In Vitro Transcription kit (Enzo Diagnostics, Inc., New York, NY). One-half of the cRNA products were purified using an RNeasy column (Qiagen Inc., Valencia, CA) and quantified with a spectrophotometer. The cRNA target ($20 \mu\text{g}$) was incubated at 94°C for 35 minutes in fragmentation buffer (Tris, magnesium acetate, potassium acetate). The fragmented cRNA was diluted in hybridization buffer (2-morpholinoethanesulfonic acid, NaCl, EDTA, Tween 20, herring sperm DNA, acetylated bovine serum albumin) containing biotin-labeled OligoB2 and Eukaryotic Hybridization Controls (Affymetrix). The hybridization cocktail was denatured at 99°C for 5 minutes, incubated at 45°C for 5 minutes and then injected into a GeneChip cartridge. The GeneChip array was incubated at 42°C for at least 16 hours in a rotating oven at 60 rpm. GeneChips were washed with a series of nonstringent (25°C) and stringent (50°C) solutions containing variable amounts of 2-morpholinoethanesulfonic acid, Tween20 and SSPE (3M NaCl, 0.2M, NaH_2PO_4 , 0.02M EDTA). The microarrays were then stained with Streptavidin Phycoerythrin and the fluorescent signal was amplified using

a biotinylated antibody solution. Fluorescent images were detected in a GeneChip® Scanner 3000 and expression data was extracted using the default settings in the MicroArray Suite 5.0 software (Affymetrix). All GeneChips were scaled to a median intensity setting of 500.

Microarray data analysis

The data were normalized by RMA and analyzed by the t-test using Partek Genomics Suite 6.4 (<http://www.partek.com/software>) (St. Louis, MO) to identify significant genes. Gene expression data after UFP exposure were compared to a control consisting of pooled data from the FA exposures (i.e. pre- and post-FA exposures). Genes with a P value of < 0.05 and a false discovery rate (FDR) of < 0.01 were considered significant. Data (CEL files) discussed in this publication have been deposited in Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) (Edgar et al., 2002) and are accessible through GEO Series accession number GSE19317.

Pathway analysis

The Ingenuity Pathways Analysis was used to map significantly altered genes with the canonical pathways (<http://www.ingenuity.com>) (Ingenuity System, Inc., Redwood City, CA). The pathways were scored by the p value from the Fisher's exact test. The pathways with a p value of < 0.05 were considered significant.

Real-time PCR

The expression of two top upregulated genes were confirmed by PCR using RNAs from 5 additional subjects exposed to FA and UFP. Total RNA from each sample was reverse transcribed into cDNA with a cDNA synthesis kit according to the manufacturer's instructions (Applied Biosystems, Foster city, CA). RNA from each sample was treated with DNaseI before cDNA synthesis under conditions described in the manual (Invitrogen, Carlsbad, CA). A total of 2 µl of cDNA (1–4 ng/µl) was mixed with the reaction mixture, which includes 10 µl of SYBR Green 2X mixture (Bio-Rad, Hercules, CA), 0.5 µl of 10 µM of the primer pair, and 7.5 µl of DNaseI-RNaseI-free water. Each sample was measured in triplicates. Bio-Rad's PCR machine iCycler (MyiQ Single Color Real-Time PCR detection System) was used to perform the PCR reactions under standard conditions. Differences in mRNA expression were reported as relative changes by comparing control values for actin with each desired gene in control and test samples. The forward and reverse primers for *CDKN1C* are CGTGGGACCTTCCCAGTA and GCTCAGCTCCTCGTGGTC, respectively. The forward and reverse primers for *SPGAP2* are GTCAAAGAGATCCGTGCTCA and AATCTCTGCCTTCTTTCGGA, respectively.

Statistical Analysis

All data were expressed as mean ± standard error (SE). Non-microarray data between FA and UFP groups was compared using the student's t-test. All statistical analyses were performed using JMP 8.0 (SAS institute, Cary, NC). A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 2611 probes (or 4.8% of the total probes) were significantly altered using the statistical algorithm. Of these, 1713 probes were annotatable with official gene symbols and proteins. There were 1020 genes with a UFP/control ratio 1.1 or –1.1 and 38 genes with a ratio >1.5 or < –1.5. These genes are shown in Table 1. We validated two upregulated genes, *CDKN1C* and *SRGAP2*, using PCR on RNAs obtained from additional 5 subjects exposed to both FA and UFP. The results confirmed the microarray data (Figure 1). We

further compared the changes in the top 10 upregulated genes (Figure 2) and top 10 downregulated genes (Figure 3) before and after exposure in FA and UFP groups. The expression of each gene after exposure to FA or UFP was normalized to its respective pre-exposure baseline. As shown in these figures, all 10 upregulated genes showed a small decrease in expression after FA while they all showed a small increase in expression after UFP. All 10 downregulated genes showed a decrease in gene expression after UFP exposure. Some of these genes, e.g., *DEFA1/DEFA3*, *ING3*, *SOD3*, had a small increase in expression after FA exposure.

We then mapped the genes with the signaling pathways and the toxicological pathways in the Ingenuity Pathway Analysis. There were 54 canonical pathways with a Fisher exact p value < 0.05 (Table 2). The top 6 signaling pathways are shown in Figure 4A and the top 6 toxicological pathways are shown in Figure 4B.

In general, inflammation-related pathways (GM-CSF signaling, IL-2 signaling, T cell receptor signaling, natural killer cell signaling, integrin signaling, B cell receptor signaling, IL-6 signaling, IL-4 signaling) and growth-related pathways (IGF-1 signaling, insulin receptor signaling, PDGF signaling, VEGF signaling, TGF- β signaling, EGF signaling, FGF signaling) were prominently represented. The IGF-1 signaling and the insulin receptor signaling are the top 2 pathways. The genes associated with these two pathways are shown in Table 3 and Table 4 respectively. They are also mapped to the pathways (Figure 5). We also noted NRF2-mediated oxidative stress response pathway, the major defense response against environmental toxicants (Li et al., 2004, Xia et al., 2006). Genes in this pathway are shown in Table 5. Other pathways that are related to host defense against environmental insults include xenobiotic metabolism signaling, role of pattern recognition receptors in recognition of bacteria and viruses and acute phase response signaling.

DISCUSSION

In this study, we tested the hypothesis that, if circulating mononuclear cells play an important role in mediating systemic effects of PM, they would show gene expression changes following exposure. Indeed, our study showed that 2-hour exposure to UFP produced mild changes in gene expression of circulating mononuclear cells 24 hours after exposure. Pathway analysis identified two insulin-related pathways (IGF-1 and insulin receptor signaling) and pathways related to host defense against environmental insults (including NRF2 signaling). The only other study that examined gene expression after controlled exposure was that by Peretz et al (Peretz et al., 2007). They also used microarray technology to profile gene expression in peripheral blood mononuclear cells in 5 healthy subjects exposed to 2 hours of diesel exhaust. They found 1290 probes (out of 54675 probes) with a diesel/air ratio of 1.5 or -1.5 at 6 or 22 hours after diesel exposure. Both studies, while using different pathway analysis programs, showed pathways involved in inflammation and oxidative stress.

The UFP in this study were used in previous controlled human exposure studies (Frampton et al., 2006, Frampton et al., 2004, Pietropaoli et al., 2004, Shah et al., 2008). The blood samples used in this study were obtained from healthy subjects who participated in a previous human exposure study (Frampton et al., 2006). This study showed that exposure of healthy or asthmatic volunteers to UFP at 50 $\mu\text{g}/\text{m}^3$ produced changes in surface markers of circulating leukocytes, including a reduction in the expression of adhesion molecules CD54 and CD18 on monocytes and CD18 and CD49d on granulocytes, and an increase in the expression of an activation marker CD25 on lymphocytes (Frampton et al., 2006). Exposure to UFP also decreased venous nitrate levels and impaired peak forearm blood flow during reactive hyperemia (Shah et al., 2008). Changes in gene expression in circulating

mononuclear cells shown in our study further support the cellular mechanisms by which UFP exposure causes systemic adverse health effects. Since more than 85% of the cells in our mononuclear cell fraction were lymphocytes, this cell type likely contributes the most changes in gene expression and may be one of the main candidate cells by which the signals from the lung are transmitted to systemic organs. Indeed, several lymphocyte-specific signaling pathways, e.g. T cell receptor signaling, natural killer cell signaling and B cell receptor signaling, were identified in our study (Table 2).

Two signaling pathways that were at the top of the list are related to insulin, i.e. IGF-1 and insulin receptor signaling. The IGF-1 signaling pathway is a potent proliferative signaling system that stimulates growth in many different tissues, including blood vessels (Abbas et al., 2008). The signal is transduced by activating the AKT signaling pathway, which was also identified by the pathway analysis in our study (Table 2). IGF-1 also inhibits programmed cell death. The insulin receptor signaling, like IGF-1 signaling, also has growth and mitogenic effects that are mostly mediated by the AKT cascade as well as by activation of the Ras/MAPK pathway. Both pathways are also important in glucose metabolism. IGF-1 lowers blood glucose while at the same time lowering serum insulin levels in normal volunteers (Moses, 2005). IGF-1 also improves insulin resistance both in type 2 diabetes and in subjects with more severe insulin resistance (Moses, 2005). Insulin receptor signaling regulates glucose and lipid metabolism that has an important role in the pathogenesis of diabetes mellitus and glucose control. Epidemiological and panel studies have linked PM exposure to diabetes mellitus. Patients with diabetes mellitus or glucose intolerance are particularly susceptible to the cardiovascular effects of ambient particle exposure (Dubowsky et al., 2006, Whitsel et al., 2009, Schneider et al., 2008, O'Neill et al., 2007, Bateson and Schwartz, 2004, Zanobetti and Schwartz, 2002). PM₁₀ exposure may contribute to the development of type 1 diabetes in children before 5 years of age (Hathout et al., 2002). PM_{2.5} exposure exaggerates insulin resistance in obese mice (Sun et al., 2009). Alterations in IGF-1 and insulin receptor signaling pathways shown in our study may provide a mechanistic link to these PM-induced effects on glucose intolerance.

Our gene expression profiling also identified pathways that are related to host defense against environmental insults include the NRF2-mediated oxidative stress response pathway, xenobiotic metabolism signaling, role of pattern recognition receptors in recognition of bacteria and viruses and acute phase response signaling. The NRF2-mediated oxidative stress response pathway is considered the Tier 1 response to environmental toxicants in the hierarchical oxidative stress hypothesis (Li et al., 2004, Xia et al., 2006). It regulates transcriptional activation of > 200 antioxidant and detoxification genes (the phase II response). The two antioxidant genes in this pathway include *GCLC*, encoding gamma-glutamyl cysteine synthetase that is the first rate limiting enzyme of glutathione synthesis, and *SOD3*, encoding extracellular SOD. All other genes appear to be related to intracellular signaling. That the many pathways related to host defense against environmental invasion was identified in circulating mononuclear cells indicates that the circulating cells likely “see” the PM-induced stress, despite the fact that they are separated by the alveolar-capillary membrane from inhaled UFP.

Several mechanisms are possible by which UFP exposure altered genes expression in circulating mononuclear cells. First, circulating mononuclear cells may be exposed directly to UFP that permeates the epithelial-endothelial barrier of the respiratory tract (Oberdorster et al., 2004, Oberdorster et al., 2002, Nemmar et al., 2001). Although some ultrafine particles have been detected in the systemic circulation, the number of particles that reaches the circulation is probably too small to cause biological effects, especially in our study in which the subjects were exposed to only 50 µg/m³ of particles for 2 hours. The second possibility is that UFP exposure recruits a new population of mononuclear cells into the

circulating pool. These cells may come from the marginated pool in the pulmonary microvasculature. This hypothesis, however, is not supported by previous observations that exposure to UFP alters peripheral blood leukocyte distribution in favor of retention in the pulmonary vascular bed (Frampton et al., 2006). The mononuclear cells (lymphocytes) may also come from the lymphatic tissues of the respiratory tracts. Carbon particles from occupational exposure are frequently detected in pulmonary lymph nodes. Cell tracer studies in the future may help unravel the kinetics of circulating mononuclear cells during PM exposure.

CONCLUSIONS

In summary, using high through-put gene expression profiling, we identified genes and pathways in peripheral blood mononuclear cells that provide plausible mechanistic links to PM-induced systemic health effects, especially those related to the cardiovascular system and glucose metabolism.

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Figure 1. RT-PCR results for *CDKN1C* and *SRGAP2*. The samples from RT-PCR were obtained from 5 additional subjects who participated in the same previous controlled exposure study. Error bars are standard deviations.

Figure 2. Comparison of expression of top 10 upregulated genes between ultrafine particle (UFP) and filtered air (Air). Data were from microarray (n = 3 each). $P < 0.05$ for all ten genes.

Figure 3. Comparison of expression of top 10 downregulated genes between ultrafine particle (UFP) and filtered air (Air). Data were from microarray (n = 3 each). $P < 0.05$ for all ten genes.

Figure 4A

Figure 4B

Figure 4.
Top 6 signaling pathways (A) and top 6 toxicological pathways (B) based on the Ingenuity Pathway Analysis.

Figure 5A

IGF-1 Signaling

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Figure 5B

Insulin Receptor Signaling

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Figure 5. Mapping of significant genes to (A) IGF-1 signaling and (B) insulin receptor signaling. Genes colored in red are upregulated and genes colored in blue are downregulated.

Table 1

Differentially expressed genes with UFP/FA ratios > 1.5 or < -1.5 in circulating mononuclear cells at 24 hours after exposure to ultrafine carbon particles.

Entrez ID	Symbol	Gene Name	Fold
1028	<i>CDKN1C</i>	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	1.86
7559	<i>ZNF12</i>	zinc finger protein 12	1.83
23380	<i>SRGAP2</i>	SLIT-ROBO Rho GTPase activating protein 2	1.82
2533	<i>FYB</i>	FYN binding protein (FYB-120/130)	1.79
149986	<i>LSM14B</i>	LSM14B, SCD6 homolog B (S. cerevisiae)	1.78
22918	<i>CD93</i>	CD93 molecule	1.76
23385	<i>NCSTN</i>	nicastatin	1.70
1848	<i>DUSP6</i>	dual specificity phosphatase 6	1.69
9555	<i>H2AFY</i>	H2A histone family, member Y	1.68
6867	<i>TACC1</i>	transforming, acidic coiled-coil containing protein 1	1.68
80005	<i>DOCK5</i>	dedicator of cytokinesis 5	1.67
10198	<i>MPHOSPH9</i>	M-phase phosphoprotein 9	1.64
9314	<i>KLF4</i>	Kruppel-like factor 4 (gut)	1.63
4659	<i>PPP1R12A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 12A	1.62
9935	<i>MAFB</i>	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	1.61
7040	<i>TGFB1</i>	transforming growth factor, beta 1	1.58
5326	<i>PLAGL2</i>	pleiomorphic adenoma gene-like 2	1.57
55665	<i>URG4</i>	up-regulated gene 4	1.55
867	<i>CBL</i>	Cas-Br-M (murine) ecotropic retroviral transforming sequence	1.54
27333	<i>GOLIM4</i>	golgi integral membrane protein 4	1.54
255488	<i>RNF144B</i>	ring finger 144B	1.54
54434	<i>SSH1</i>	slingshot homolog 1 (Drosophila)	1.54
7494	<i>XBP1</i>	X-box binding protein 1	1.54
3192	<i>HNRNPU</i>	heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	1.53
84548	<i>TMEM185A</i>	transmembrane protein 185A	1.53
5780	<i>PTPN9</i>	protein tyrosine phosphatase, non-receptor type 9	1.52
1508	<i>CTSB</i>	cathepsin B	1.51
7266	<i>DNAJC7</i>	DnaJ (Hsp40) homolog, subfamily C, member 7	1.51
4194	<i>MDM4</i>	Mdm4 p53 binding protein homolog (mouse)	1.51
9414	<i>TJP2</i>	tight junction protein 2 (zona occludens 2)	1.51
7357	<i>UGCG</i>	UDP-glucose ceramide glucosyltransferase	1.51
10019	<i>SH2B3</i>	SH2B adaptor protein 3	1.50
8676	<i>STX11</i>	syntaxin 11	1.50
254065	<i>BRWD3</i>	bromodomain and WD repeat domain containing 3	-1.51
57560	<i>IFT80</i>	intraflagellar transport 80 homolog (Chlamydomonas)	-1.52
54556	<i>ING3</i>	inhibitor of growth family, member 3	-1.81
13216	<i>DEFA1//DEFA3</i>	defensin, alpha 1//defensin, alpha 3, neutrophil-specific	-1.82
54205	<i>CYCS</i>	cytochrome c, somatic	-1.97

Table 2

Ingenuity signaling and toxicological pathways associated with UFP exposure. Only pathways with a p value of < 0.05 (Fisher exact test) are shown.

Ingenuity canonical pathways	Number of genes in the pathway	P-value
IGF-1 signaling	16	0.0000
Insulin receptor signaling	19	0.0000
PI3K/AKT signaling	16	0.0000
JAK/Stat signaling	11	0.0001
GM-CSF signaling	11	0.0001
IL-2 signaling	10	0.0001
Neurotrophin/TRK signaling	11	0.0001
Ceramide signaling	12	0.0002
PTEN signaling	13	0.0002
Erythropoietin signaling	11	0.0002
Cardiac β -adrenergic signaling	15	0.0002
T cell receptor signaling	13	0.0003
Natural killer cell signaling	13	0.0003
Dopamine receptor signaling	11	0.0004
BMP signaling pathway	11	0.0006
Amyotrophic lateral sclerosis signaling	12	0.0007
Actin cytoskeleton signaling	20	0.0008
Integrin signaling	19	0.0008
G-Protein coupled receptor signaling	19	0.0011
PDGF signaling	10	0.0011
VEGF signaling	11	0.0012
Axonal guidance signaling	29	0.0018
SAPK/JNK signaling	11	0.0020
ERK/MAPK signaling	17	0.0022
TGF- β signaling	10	0.0023
EGF signaling	7	0.0032
cAMP-mediated signaling	15	0.0034
FGF signaling	10	0.0038
Tight junction signaling	15	0.0039
B cell receptor signaling	14	0.0048
Xenobiotic metabolism signaling	19	0.0048
Glucocorticoid receptor signaling	20	0.0076
VDR/RXR activation	9	0.0083
Role of pattern recognition receptors in recognition of bacteria and viruses	9	0.0083
Macropinocytosis	8	0.0089
Wnt/ β -catenin signaling	14	0.0105
Fc epsilon RI signaling	10	0.0110
Hepatic fibrosis/Hepatic stellate cell activation	12	0.0129

Ingenuity canonical pathways	Number of genes in the pathway	P-value
Synaptic long term potentiation	10	0.0155
Apoptosis signaling	9	0.0155
Glycosphingolipid biosynthesis - lactoseries	3	0.0166
IL-6 signaling	9	0.0204
NRF2-mediated oxidative stress response	14	0.0214
IL-4 signaling	7	0.0257
Calcium signaling	13	0.0355
PPAR signaling	8	0.0380
Acute phase response signaling	13	0.0380
Sonic hedgehog signaling	4	0.0398
Neuregulin signaling	8	0.0407
Ephrin receptor signaling	13	0.0417
14-3-3-mediated signaling	12	0.0417
PPAR α /RXR α activation	12	0.0437
Huntington's disease signaling	15	0.0447

Table 3

Genes in IGF-1 signaling altered by UFP exposure.

Entrez ID	Symbol	Gene Name	Fold
1457	<i>CSNK2A1</i>	casein kinase 2, alpha 1 polypeptide	-1.26
2885	<i>GRB2</i>	growth factor receptor-bound protein 2	1.25
3479	<i>IGF1</i>	insulin-like growth factor 1 (somatomedin C)	-1.23
3486	<i>IGFBP3</i>	insulin-like growth factor binding protein 3	1.12
3488	<i>IGFBP5</i>	insulin-like growth factor binding protein 5	1.12
22808	<i>MRAS</i>	muscle RAS oncogene homolog	1.27
5170	<i>PDPK1</i>	3-phosphoinositide dependent protein kinase-1	1.21
5286	<i>PIK3C2A</i>	phosphoinositide-3-kinase, class 2, alpha polypeptide	-1.13
5289	<i>PIK3C3</i>	phosphoinositide-3-kinase, class 3	-1.14
5567	<i>PRKACB</i>	protein kinase, cAMP-dependent, catalytic, beta	-1.44
5576	<i>PRKAR2A</i>	protein kinase, cAMP-dependent, regulatory, type II, alpha	1.24
5781	<i>PTPN11</i>	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	1.44
5894	<i>RAF1</i>	v-raf-1 murine leukemia viral oncogene homolog 1	1.17
6237	<i>RRAS</i>	related RAS viral (r-ras) oncogene homolog	1.33
6464	<i>SHC1</i>	SHC (Src homology 2 domain containing) transforming protein 1	-1.34
6655	<i>SOS2</i>	son of sevenless homolog 2 (Drosophila)	-1.18

Table 4

Genes in insulin receptor signaling altered by UFP exposure.

Entrez ID	Symbol	Gene Name	Fold
867	<i>CBL</i>	Cas-Br-M (murine) ecotropic retroviral transforming sequence	1.54
2885	<i>GRB2</i>	growth factor receptor-bound protein 2	1.25
2931	<i>GSK3A</i>	glycogen synthase kinase 3 alpha	-1.15
22808	<i>MRAS</i>	muscle RAS oncogene homolog	1.27
4690	<i>NCK1</i>	NCK adaptor protein 1	1.18
5170	<i>PDPK1</i>	3-phosphoinositide dependent protein kinase-1	1.21
5286	<i>PIK3C2A</i>	phosphoinositide-3-kinase, class 2, alpha polypeptide	-1.13
5289	<i>PIK3C3</i>	phosphoinositide-3-kinase, class 3	-1.14
4659	<i>PPP1R12A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 12A	1.62
81706	<i>PPP1R14C</i>	protein phosphatase 1, regulatory (inhibitor) subunit 14C	-1.33
5567	<i>PRKACB</i>	protein kinase, cAMP-dependent, catalytic, beta	-1.44
5576	<i>PRKAR2A</i>	protein kinase, cAMP-dependent, regulatory, type II, alpha	1.24
5770	<i>PTPN1</i>	protein tyrosine phosphatase, non-receptor type 1	-1.15
5781	<i>PTPN11</i>	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	1.44
5894	<i>RAF1</i>	v-raf-1 murine leukemia viral oncogene homolog 1	1.17
23433	<i>RHOQ</i>	ras homolog gene family, member Q	1.26
6237	<i>RRAS</i>	related RAS viral (r-ras) oncogene homolog	1.33
6464	<i>SHC1</i>	SHC (Src homology 2 domain containing) transforming protein 1	-1.34
6655	<i>SOS2</i>	son of sevenless homolog 2 (Drosophila)	-1.18

Table 5

Genes in the NRF2-mediated oxidative stress response pathway

Entrez ID	Symbol	Gene Name	Fold
7266	<i>DNAJC7</i>	DnaJ (Hsp40) homolog, subfamily C, member 7	1.51
4217	<i>MAP3K5</i>	Mitogen-activated protein kinase kinase kinase 5	1.36
6237	<i>RRAS</i>	Related RAS viral (r-ras) oncogene homolog	1.33
22808	<i>MRAS</i>	Muscle RAS oncogene homolog	1.27
54431	<i>DNAJC10</i>	DnaJ (Hsp40) homolog, subfamily C, member 10	1.25
2729	<i>GCLC</i>	Glutamate-cysteine ligase, catalytic subunit	1.19
5894	<i>RAF1</i>	v-raf-1 murine leukemia viral oncogene homolog 1	1.17
7415	<i>VCP</i>	Valosin-containing protein	1.16
5286	<i>PIK3C2A</i>	Phosphoinositide-3-kinase, class 2, alpha polypeptide	-1.13
54788	<i>DNAJB12</i>	DnaJ (Hsp40) homolog, subfamily B, member 12	-1.14
5289	<i>PIK3C3</i>	Phosphoinositide-3-kinase, class 3	-1.14
3727	<i>JUND</i>	Jun D proto-oncogene	-1.19
8878	<i>SQSTM1</i>	Sequestosome 1	-1.27
6649	<i>SOD3</i>	Superoxide dismutase 3, extracellular	-1.42