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REVIEW

Alterations of gene expression profiles induced by sulfur dioxide in rat lungs

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Abstract Sulfur dioxide (SO_2) is a ubiquitous air pollutant, presents in low concentrations in urban air and in higher concentrations in working environment. Few data are available on the effects of being exposed to this pollutant on the molecular mechanism, although some biochemical changes in lipid metabolism, intermediary metabolism and oxidative stress have been detected. The present investigation aimed at analyzing the gene expression profiles of the lungs of Wistar rats short-term (20 ppm, 6 h/day, for seven days) and long-term (5 ppm, 1 h/day, for 30 days) exposed to SO_2 by Affymetrix GeneChip (RAE230A) analysis. It was found that 31 genes, containing 18 known genes and 13 novel genes, were up-regulated, and 31 genes, containing 20 known genes and 11 novel genes, were down-regulated in rats short-term exposed to SO_2 compared with control rats. While there were 176 genes, containing 82 known genes and 94 novel genes, were up-regulated, and 85 genes, containing 46 known genes and 39 novel genes, were down-regulated in rats long-term exposed to SO_2 compared with control rats. It is suggested that: (1) SO_2 exerts its effects by different mechanisms in vivo at high-dose short-term inhalation and at low-dose long-term inhalation; (2) a notable feature of the gene expression profile was the decreased expression of genes related to oxidative phosphorylation in lungs of rats short-term exposed to SO_2, which shows high-dose short-term exposed to SO_2 may cause the deterioration of mitochondrial functions; (3) discriminating genes in lungs of rats long-term exposed to SO_2 included those involved in fatty acid metabolism, immune, inflammatory, oxidative stress, oncogene, tumor suppresser and extracellular matrix. The mechanism of low-dose long-term exposed to SO_2 is more complex.

Keywords sulfur dioxide, lung, rat, GeneChip analysis, gene expression

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1 Introduction

Sulfur dioxide (SO_2) is a common air pollutant released into the atmosphere from the combustion of fossil fuel (Rall, 1974), presents in low concentration in the community air as well as in higher concentration in some work places. Inhaled SO_2 can easily be hydrated to produce sulfurous acid in the respiratory tract, which subsequently dissociates to form its derivatives, bisulfite and sulfite (1 : 3 M/M, in neutral fluid) (Shapiro, 1977). The derivatives can be absorbed into blood or other body fluid. It is generally considered that SO_2 and its vivo bisulfite/sulfite derivatives are toxic to the respiratory system and can cause allergic reactions, the most common of which is bronchoconstriction in asthmatics (Ceballos-Picot et al., 1992), it also can act as a comutagen and cocarcinogen to link to lung cancer (Reed et al., 1986; Lester, 1995; Meng and Zhang, 1999). However, SO_2 inhalation may induce chromosomal aberrations (CA), micronuclei (MN) in mouse bone-marrow cells (Meng et al., 2002; Meng and Zhang, 2002). SO_2 and its vivo derivatives may also induce CA, MN and sister chromatid exchanges (SCE) in human lymphocytes (Meng and Zhang, 1990; 1992). Recent studies have found that the derivatives can cause changes of sodium currents and transient outward potassium currents in the hippocampal CA1 neurons and dorsal root ganglion neurons from rats (Meng and Sang, 2002; Du and Meng 2004a; Du and Meng 2004b). SO_2 inhalation may cause changes of oxidative stress and antioxidation status in various organs of mice and brains of rats and guinea pigs and the erythrocytes of rats (Haider et al., 1981; Yargicoglu et al., 1999; Meng, 2003). SO_2 pollution may play an independent role in triggering ischemic cardiac events (Sunyer et al., 2003). It is reported that c-jun mRNA was expressed in alveolar macrophages from rats exposed to air containing 100 mg/m^3 volcanic ash with or without 1.5 ppm SO_2 (Samukawa et al., 2003). Recently, Meng et al. have reported that SO_2 and its deriratives are the systemic toxic agent, can cause DNA damage and oxidative damage in multiple organs from mice, not only lungs (Meng et al., 2004; Meng, 2003).

However, little information is available about the mechanism of SO_2 or bisulfite/sulfite toxicity. It may involve oxidation damage in cells, tissues, and organs, caused by sulfur- and oxygen-centered free radicals formed in the process of sulfite oxidation (Shi, 1994; Shi and Mao, 1994). Lipid peroxidation now is considered as a new mechanism of SO_2-induced damage (Meng, 2003; Koksal et al., 2003). In the present work, to understand molecular events associated with SO_2 inhalation in mammalian animals, we analyzed the gene expression profiles of the lungs of rats short-term or long-term exposed to SO_2 by Affymetrix GeneChip (RAE230A) analysis.

2 Materials and methods

2.1 Animals and treatment

Male Wistar rats weighing between 180 and 200 g were used for the present experiment. Rats were housed in groups of three in metallic cages under standard condition (24°C ± 2°C and 50% ± 5% humidity) with a 12-h light-dark cycle. The rats had access to food and water ad libitum. The rats were randomly divided into four groups, each groups consisting of three animals, two groups were exposed to SO_2, and two groups served as corresponding controls. The exposure protocol was of two types, one for examining short-term effects and the other for investigating long-term effects. In the short-term exposure protocol, the group of rats received SO_2 inhalation exposure at 56 mg/m^3 in 1-m^3 exposure chambers for 6 h per day (9 : 00 a.m.–3 : 00 p.m.) for seven days. In the long-term exposure protocol, the rats were exposed to SO_2 at 14 mg/m^3 in an identical chamber for 1 h per day (9 : 00–10 : 00 a.m.) for 30 days. The corresponding control groups were exposed to filtered air in identical chambers for the same period of time. Each chamber was fitted with one entry tube at the upper level and one exhaust tube at the lower level. The SO_2 gas was delivered continuously to rats through the entry tube and distributed evenly by a fan, while the air pressure in the chamber permitted the gas to exit through the exhaust tube at the lower level. Fresh air at the intake port diluted the SO_2 to yield the desired concentrations within the chamber, which were monitored by measurement every 30 min by pararosaniline hydrochloride spectrophotometry (Goyal, 2001).

Twenty-four hours after the last exposure, tissues were removed and quick frozen for later microarray analysis. All animal procedures were approved by the Shaanxi University Animal Investigational Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by Ministry of Health People's Republic of China.

2.2 Microarray analysis

RNA preparation and analysis were done according to the Affymetrix Inc. protocol. Briefly, the total RNA was isolated from lungs of rats by using TRIzol Reagent (Invitrogen Life Technologies, P/N 15596-018) and purified by RNeasy Mini Kit (QIAGEN, P/N 74104) according to the manufacturer's protocols. RNA was eluted in nuclease-free water and stored at –80°C. The first strand of cDNA was synthesized from equal amounts of total RNA by using the SuperScript II reverse transcriptase (Invitrogen Life Technologies, P/N 18064-014) with an T7– Oligo(dT)24 Primer. The sequence was 5′-GGCCAGTGAATTGTAATACGACTCACTATAGG-GAGGCGG- (dT)24-3′. After the second strand synthesis, the cDNA was phenol/chloroform extracted and ethanol precipitated.

Following this, biotinylated cRNA was synthesized from the cDNA using the Enzo RNA Transcript Labeling Kit (Affymetrix, P/N 900182) according to the manufacturer's instructions. Approximately 20 μg of cRNA was then fragmented in a solution of 40 mM Tris–acetate, pH 8.1, 100 mM KOAc and 30 mM MgOAc at 94°C for 35 min.

Labeled cRNA was hybridized to the Affymetrix GeneChip Test 3 Array to verify the quality of labeled cRNA. Following this, cRNA was hybridized to the Affymetrix GeneChip Rat Expression RAE230A array (Affymetrix, P/N 3005333). The cRNA was hybridized overnight at 45°C using a Hybridization Oven 640 (Affymetrix). Rat RAE230A array was washed and stained using a Fluidics Station 400 (Affymetrix). Each hybridized RAE230A array was scanned with a G2500AGeneArray Scanner (Affymetrix) and analyzed with Microarray Suite Version 5.0 (Affymetrix).

2.3 Data analysis

Reproducible results could be got by analyzing GeneChip analysis from one experiment, because 11 pairs of 25-mer oligonucleotide probes, including a perfect match probe, which is a oligonucleotide designed to be complementary to a reference sequence and mismatch probe, which is a oligonucleotide designed to be complementary to a reference sequence except for a single base change at the 13th position, are used to measure the level of transcription of each sequence represented on the GeneChip. To minimize individual variation, lungs taken from three treatments or three control rats in each group were separately homogenated, from which total RNA were isolated.

Each RNA sample was converted to cDNA followed by cRNA synthesis, hybridization, and chip scanning. Nine-pair comparison analysis was performed to identify differentially regulated genes in short-term or long-term group, using the expression of their corresponding controls as baselines by Affymetrix MAS5.0 software.

Probe sets that were assigned by MAS 5.0 as "absent" were filtered out in all six arrays or assigned as "no change" in all nine-pair comparisons in each treatment. It was identified that genes, the expression of which changed significantly in each treatment according to the criterion that at least six of the nine-pair comparisons had a concordant

change in gene expression that was greater than 50% after each treatment. Similar criterion was applied by Cheok et al. (2003).

3 Results and discussion

To test whether SO_2 affects the gene expression in lung, and if any, to search for the molecular basis underlying pulmonary responses against expose to SO_2, we performed microarray analysis of gene expression profiles in lungs of rats exposed to SO_2 at 20 ppm for 6 h daily in seven consecutive days in short-term treatment group or at 5 ppm for 1 h daily in 30 consecutive days in long-term treatment group.

3.1 Discriminating genes in lungs of rats short-term exposed to SO_2 (Table 1)

The number of up-regulated genes in short-term treatment was 31genes, including 18 known genes and 13 expressed sequence tags (ESTs). The number of down-regulated genes in short term treatment was 30 genes, including 19 known genes and 11 novel genes that included one gene with unknown function and 10 ESTs. Table 1shows all the known discriminating genes in lungs of rats short-term exposed to SO_2.

3.1.1 Electron transport

Several genes associated to electron transport were changed in rats short-term exposed to SO_2 compared with control rats. ATPase inhibitors (rat mitochondrial IF1 protein) were up-regulated to 4.8, 5.9 and 4.3 times the level in control rats, respectively (Table 1-*1-3). The ATPase inhibitor binds the beta subunit of the (F_1F_0) H^+-ATPase, which can function reversibly, by the synthesis of ATP as an ATP synthase. Cytochrome c oxidase, which is reported decrease in oxidative stress in insects (Schwarze et al., 1998) as well as mammals (Navarro et al., 2002), was down-regulated 2.5 fold (Table 1-*4). It is already reported that (F_1F_0) H^+-ATPase activity of *Oenococcus oeni* is decreased by SO_2 (Carreté et al., 2002). However, no report is available on ATPase and cytochrome *c* oxidase in mammals exposed to SO_2.

Previous study shows that defects in the activities of the respiratory complexes IV (cytochrome *c* oxidase, COX) may lead to inhibition of the mitochondrial ATP-synthase enzyme in vivo. The ATP-synthase activity is down-regulated under anoxic conditions in fibroblasts from healthy individuals, presumably due to a reduction of the potential across the inner mitochondrial membrane. Similarly, in fibroblasts from children with a defect in the respiratory chain enzymes IV the electron transport across the respiratory chain is impaired which results in a drop of the membrane potential. This in turn may lead to (membrane potential-dependent) binding of IF1 to the ATP-synthase molecule and finally a secondary inhibition of the mitochondrial ATP-synthase activity. It has been identified several patients who showed defects in the complex IV activities who had a decreased capacity of the mitochondrial ATP-synthase and abnormal regulation of the enzyme in vivo (Das, 2003).

Deficiency of mitochondrial cytochrome c oxidase in senescent cells enhances oxidative stress (Xin et al., 2003). In various defects of respiratory chain complexes increased superoxide production as well as overproduction of other reactive oxygen species (ROS) has been found (Esposito et al., 1999; Albers and Beal, 2000; Sayen et al., 2003). Fibroblasts harboring the NARP (neuropathy, ataxia, retinitis pigmentosa)—mutation of the ATP—synthase have been shown to produce excess amounts of superoxide (Geromel et al., 2002). The overproduction of superoxide and other ROS probably causes further cell damage and mutations of mtDNA finally resulting in cell death (Esposito et al., 1999). Several studies have found that SO_2 inhalation enhances lipid peroxidation in the brains of rats and guinea pigs and in the rat erythroctes and nine organs of mice (Haider et al., 1981; Etlik et al., 1995; Gumuslu et al., 1998; Yargicoglu et al., 1999; Meng, 2003). Mitochondrial swelling and decrease in oxidative phosphorylation (Utsumi et al., 1965), release of hydrolytic enzymes from lysosomes (Wills and Wilkinson, 1966), and change in endoplasmic reticulum (Bidlack and Tappel, 1974) all have been described as biochemical distortions following lipid peroxidation.

These results suggest that SO_2 inhalation can modulate oxidative phosphorylation by altering the activities of ATPase and cytochrome c oxidase. Together, these changes will possibly lead to a lowering in the influx of reductor power to the mitochondrial electron transport chain thereby potentially leading to an ATP/ADP balance change that may compromise muscle function. Troponin 1, type 3 (Tnni3), troponin T2 (Tnnt2), myosin heavy chain, polypeptide 6 (Myh6), tropomyosin 1, alpha (Tpm1), tropomyosin 1, alpha (Tpm1) and actin alpha cardiac 1 (Actc1), which are muscle components, were down-regulated at different levels (Table 1-*5-11), also suggesting that the deterioration of mitochondrial functions in the lungs of rats exposed to SO_2.

3.1.2 Thyroid hormone

Expressions of several genes associated to thyroid hormone were changed in rats short-term exposed to SO_2 compared with control rats. Transthyretin (Ttr) is a kind of thyroid hormone-binding protein and probably transports thyroxin from the bloodstream to the brain (Table 1-*12). Nuclear receptor subfamily 1, group D, member 1 (Nr1d1) is a possible receptor for triiodothyronine (T3) (Table 1-*13). They were up-regulated 2.3 fold. While the thyroid hormone responsive protein (THRP), which is a novel gene product that remains responsive to thyroid hormone in the cerebral cortex of adult rats, was down-regulated (Table 1-*14-15).

Table 1 Discriminating genes in lungs of rats short-term exposed to SO_2 at 20 ppm

Classification	Accession no.	Gene name	FC	
Transport	gb: NM_134326.1	Albumin (Alb)	3.7	a
	gb: NM_012681.1	Transthyretin (Ttr)	2.3	*12
	gb: NM_053365.1	Fatty acid binding protein 4 (Fabp4)	–4.7	b
	gb: NM_024162.1	Fatty acid binding protein 3 (Fabp3)	–3.8	b
Electron transport	gb: AF368860.1	ATPase inhibitor	4.8	*1
	gb: AF198441.1	ATPase inhibitor	5.9	*2
	gb: AA893518	Rattus norvegicus similar to Urinary protein 3 Precursor (RUP-3) (LOC300504), mRNA	4.3	*3
	gb: NM_012812.1	Cytochrome *c* oxidase, subunit VIa, polypeptide 2 (Cox6a2)	–2.5	*4b
Transcription	gb: M25804.1	Nuclear receptor subfamily 1, group D, member 1 (Nr1d1)	2.3	*13
	gb: NM_031345.1	Glucocorticoid-induced leucine zipper (Gilz)	1.5	
	gb: NM_053536.1	Kruppel-like factor 15(Klf15)	1.6	
Inflammatory response	gb: BF289368	Lipopolysaccharide binding protein (Lbp)	1.6	
	gb: D88586.1	Eosinophil cationic protein (ECP)	2.9	b
	gb: AF053312.1	Chemokine (C-C motif) lignad 20 (Ccl20)	–1.8	
	gb: AF245172.1	Guanine deaminase (Gda)	–1.7	b
Immune	gb: BG374683	Rat Ig active lambda2-like chain mRNA, 3′ end	1.6	
	gb: AI029631	Rat Ig active lambda2-like chain mRNA, 3′ end	1.8	
	gb: L22654.1	Rat anti-acetylcholine receptor antibody gene, rearranged Ig gamma-2a chain, VDJC region, complete cds	–2.1	
Microtubule	gb: NM_017204.1	Microtubule-associated protein 6 (Mpat6)	1.7	
Phase 1 reactions enzyme	gb: NM_053433.1	Flavin-containing monooxygenase 3 (Fmo3)	1.7	
	gb: U09742.1	Testosterone 6-beta-hydroxylase (CYP3A2)	3.0	
	gb: NM_012542.1	Cytochrome P450, subfamily IIA (phenobarbital-inducble)/ (Cytochrome P450 IIA3) (CYP2A3a)	–1.9	b
	gb: X00469.1	Cytochrome P450, 1a1 (CYP1A1)	–1.7	
Signal transduction	gb: NM_012903.1	Acidic nuclear phosphoprotein 32 family, member A (Anp32a)	1.6	
	gb: AI598401	Proviral integration site 1 (Pim1)	2.1	a
	gb: BE110108	Protein tyrosine phosphatase, non-receptor type 16 (Ptpn16)	1.6	
Calcium ion binding	gb: NM_017309.1	Protein phospatase 3, regulatory subunit B, alpha isoform, type 1 (Ppp3r1)	1.8	a
Cell-cell signaling	gb: NM_031135.1	TGFB inducible early growth response (Tieg)	1.6	
Fatty Acid Synthesis	gb: J02585.1	Stearoyl-Coenzyme A desaturase 1 (Scd1)	–2.6	b
Hormone	gb: NM_012703.1	Thyroid hormone responsive protein (Thrsp)	–9.1	*14b
	gb: AI169092	Thyroid hormone responsive protein (Thrsp)	–6.5	*15
Muscle	gb: NM_017144.1	Troponin 1, type 3 (Tnni3)	–6.4	*5b
	gb: NM_012676.1	Troponin T2 (Tnnt2)	–5.8	*6b
	gb: NM_017239.1	Myosin heavy chain, polypeptide 6 (Myh6)	–4.2	*7b
	gb: AF370889.1	Tropomyosin 1, alpha(Tpm1)	–2.7	*8b
	gb: NM_019131.1	Tropomyosin 1, alpha (Tpm1)	–2.4	*9b
	gb: AA800705	Actin alpha cardiac 1 (Actc1)	–1.5	*10b
	gb: NM_057144.1	Cysteine-rich protein 3 (Csrp3)	–8.0	*11b
Antioxidant	gb: AB030829.1	Carbonic anhydrase 3 (Ca3)	–4.4	b
	gb: NM_019292.1	Carbonic anhydrase 3 (Ca3)	–3.4	
Metabolism	gb: BI277460	Phosphoenolpyruvate carboxykinase, cytosolic (GTP) (PCK)	–2.7	
Extracellular matrix	gb: AI230238	Procollagen, type X, alpha 1 (Col10a1)	–2.4	b

Note: FC: fold of change (SO_2 exposure group/control).
*1–15: the genes those are described in Section 3.
a) the genes which their expressions in both short- and long-term groups were changed in the same direction (up- or down-regulated).
b) the genes which their expressions in both short- and long-term groups were changed in different direction (up- and down-regulated).

The biological effects of THRP are currently unknown. It may function as a homeostatic adaptive response during protein deficiency (Lee et al., 2002). THRP expression induces PC12 cell death and the effect of T3 treatment on PC12 cell survival may be mediated by THRP (Haas et al., 2003). These results suggest that SO_2 might cause some changes of the physiological processes of thyroid hormone in vivo.

3.2 Discriminating genes in lungs of rats exposed to SO_2 for 30 days (Table 2)

The number of up-regulated genes in long-term treatment was 173genes, including 79 known genes and 94 novel genes that included 20 genes with unknown function and 74 ESTs. The number of down-regulated genes in long-term treatment was 85 genes, including 46 known genes and 39 novel genes that

included five genes with unknown function and 34 ESTs. Table 2 shows that all the known discriminating genes in lungs of rats were long-term exposed to SO_2.

3.2.1 Fatty acid synthesis and oxidation

Our first focus was on the genes encoding enzymes involved in fatty acid metabolism. They are involved either in fatty acid synthesis: ATP citrate-lyase (*Acly*), acetyl-CoA carboxylase (*Acac*), fatty acid synthase (*Fasn*), malic enzyme (*Me*), or in fatty acid desaturation and secretion: Stearoyl-CoA desaturase 1 (*Scd1*) (Table 2-*1-10). These genes are known to be regulated in a co-ordinated manner under the influence of various nutrients and hormones (Jump et al., 1994; Towle et al., 1996; Jump and Clarke, 1999). In the present experiment, they were all coordinated up regulation in lungs of rats long-term exposed to SO_2.

Recent studies suggest that CCAAT/enhancing-binding protein (C/EBP) isoforms and sterol regulatory element binding factor (Srebf)-1c regulate fatty acid synthesis in adult type II cells in vitro (Zhang et al., 2004). In the present study, Srebf1 was up-regulated 1.7 fold, while C/EBP delta was down-regulated 2.1 fold(Table 2-*11-12). These results show that the mRNA levels of enzymes of fatty acid synthesis appear to be regulated by SREBF1 in lungs of rats long-term exposed to SO_2.

In the cytoplasm, fatty acid synthesis is controlled via Acly, Acac and Fasn. The Acly reaction enhances fatty acid synthesis by providing more acetyl CoA and NADPH substrates and enhancing Acac activity. The malonyl CoA generated in the Acac reaction then inhibits fatty acid oxidation by reversibly inhibiting carnitine palmitoyltransferase I (Cpt1). It is the first committed intermediate in FA synthesis and is an allosteric inhibitor of Cpt1. Cpt1 is the rate-limiting enzyme in FA oxidation (McGarry et al., 1989). Fatty acid oxidation might be suppressed in lungs of rats long-term exposed to SO_2, though its two isoforms were changed in different direction (Table 2-*13-14).

3.2.2 Immune, inflammatory and oxidative stress

Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha (Nfkbia), which is one of the three main inhibitory proteins of nuclear factor kappa B (NF-κB), was down-regulated in lungs of rats long-term exposed to SO_2 (Table 2-*15). In unstimulated cells, NF-κB exists in an inactive state in the cytoplasm complexed to the inhibitory IκB molecules. Upon stimulation, IκB is rapidly degraded, freeing NF-κB to translocate to the nucleus and to activate the expression of its target genes (Whiteside and Israel, 1997). Cellular enrichment with polyunsaturated fatty acids can induce the development of oxidative stress condition and the activity of activating protein-1 (AP-1) and NF-κB (Maziere et al., 1999). NF-κB transcription factors are a family of structurally related eukaryotic transcription factors that are involved in the control of a vast array of processes, such as immune and inflammatory responses, developmental processes, cellular growth and programmed cell death (apoptosis). In addition, these factors are active in a number of disease states, including cancer, arthritis, inflammation, asthma, neurodegenerative diseases and cardiovascular abnormalities. This suggests that NF-kappa B activation in lungs may play an important role in the initiation and progression of SO_2-induced pulmonary inflammation and cellular damages.

Major histocompatibility complex (MHC class I and II) was directly targeted by NF-κB (Haddad, 2002) (Table 2-*16-21). They were suppressed by long-term SO_2 inhalation. The immune system can be impaired (immunosuppression) leading to an increased chance of developing an infection or infrequently, an increased risk of developing cancer.

Ca^{2+}-dependent phospholipase A2 ($cPLA_2$) also belongs to NF-κB target genes (Rossi et al., 2000). It was up-regulated in lungs of rats long-term exposed to SO_2 (Table 2-*22). In humans, PLA_2s are associated with numerous clinical inflammatory processes (Nevalainen et al., 2000). PLA_2s are present as membrane-associated and soluble enzymes in almost all cell types and play important roles in the biosynthesis of eicosanoids, turnover of membrane phospholipids, cellular signalling and protection of membranes against peroxidation damage (van Kuijk et al., 1987; Balsinde et al., 2002).

It has already been reported that SO_2 or bisufite/sulfite can lead to lipid peroxidation, oxidative stress and antioxidation status in multiple organs of mammals (Haider et al., 1981; Yargicoglu et al., 1999; Meng, 2003). In the present study, carbonic anhydrase 3 (Ca3) and peroxiredoxin 6 (Prdx6) were up-regulated while ceruloplasmin (Cp) was down-regulated, although the expression levels of Cu, Zn-SOD and Se-dependent GSH-Px, the activities of which decrease in mice by SO_2 inhalation, in rats exposed to SO_2 were the same as the levels in control rats (Table 2-*23-26).

Cp is a 135 kDa protein, the principal carrier of copper in plasma, which plays an important role in iron homeostasis and is also an effective anti-oxidant for a variety of free radicals. It acts as a ferroxidase and superoxide dismutase (Floris et al., 2000). Ca3 may provide protection from oxidative damage, and the lower levels of free radicals in cells overexpressing Ca3 may also affect growth signalling pathways (Saarnio, 2000). Prdx6, also termed antioxidant protein-2 or 1-cys peroxiredoxin, contains a single redox-active cysteine (Cys^{47}) and is a cytosolic bifunctional enzyme with non-selenium glutathione peroxidase and PLA_2 activities (Choi et al., 1998; Kang et al., 1998; Chen et al., 2000). Lipid hydroperoxides are hydrolyzed by PLA_2 to free fatty acid hydroperoxides and then converted into a variety of toxic end-products such as aldehydes and epoxides. Thus, Prdx6 could promote restoration of membrane function and integrity by cleavage and elimination of peroxidized phospholipids by combining its peroxidase and PLA_2

Table 2 Discriminating genes in lungs of rats exposed to SO_2 at 5 ppm for 30 days

Classification	Accession no.	Gene name	FC	
Fatty Acid Synthesis	gb: NM_016987.1	ATP citrate lyase(Acly)	2.0	*1
	gb: BI296153	Acetyl-coenzyme A carboxylase (Acac)	1.6	*2
	gb: NM_022193.1	Acetyl-coenzyme A carboxylase (Acac)	1.6	*3
	gb: NM_017332.1	Fatty acid synthase (Fasn)	2.3	*4
	gb: AI179334	Fatty acid synthase (Fasn)	2.4	*5
	gb: NM_012600.1	Malic enzyme 1 (Me1)	2.6	*6
	gb: M30596.1	Malic enzyme 1 (Me1)	3.0	*7
	gb: J02585.1	Stearoyl-Coenzyme A desaturase 1 (Scd1)	5.8	*8b
	gb: BE107760	Stearoyl-Coenzyme A desaturase 2 (Scd2)	2.3	*9
	gb: NM_031841.1	Stearoyl-Coenzyme A desaturase 2 (Scd2)	2.0	*10
	gb: D90109.1	Fatty acid Coenzyme A ligase, long chain 2	2.7	
	gb: BI277523	Fatty acid Coenzyme A ligase, long chain 2	2.0	
Fatty Acid Oxidation	gb: NM_031559.1	Carnitine palmitoyltransferase 1, liver (Cpt1a)	–1.7	*13
	gb: NM_013200.1	Carnitine palmitoyltransferase 1 b	1.9	*14
Immune response	gb: NM_012645.1	RT1 class Ib gene(Aw2) (RT1Aw2)	–1.7	*16
	gb: AJ243338.1	RT1 class Ib gene(Aw2) (RT1Aw2)	–1.7	*17
	gb: M24026.1	RT1 class Ib gene(Aw2) (RT1Aw2)	–1.5	*18
	gb: L40364.1	RT1 class Ib gene(Aw2) (RT1Aw2)	–1.6	*19
	gb: NM_012646.1	RT1 class Ib gene, H2-TL-like, grc region (N1) (RT1-N1)	–2.0	*20
	gb: BM389513	Rat mRNA for MHC class II antigen RT1.B-1 beta-chain	–1.8	*21
	gb: NM_053373.1	Peptidoglycan recognition protein (Pglyrp)	–2.0	
Inflammatory response	gb: AF245172.1	Guanine deaminase (Gda)	1.7	b
	gb: AI234860	Phospholipase A2, group IB (Pla2g1b)	1.7	*22
	gb: D88586.1	Eosinophil cationic protein (ECP)	–1.6	b
Antioxidant	gb: AB030829.1	Carbonic anhydrase 3 (Ca3)	2.0	*23b
	gb: AF014009.1	Peroxiredoxin 6 (Prdx6)	1.6	*24
	gb: AF202115.1	Ceruloplasmin (CP)	–1.9	*25
	gb: NM_012532.1	Ceruloplasmin (CP)	–1.7	*26
Oncogene	gb: BF415939	c-fos oncogene (c-fos)	2.0	*27
	gb: NM_133397.1	v-ets erythroblastosis virus E26 oncogene like (avian) (Erg)	1.6	*28
	gb: BI288619	v-jun sarcoma virus 17 oncogene homolog (avian) (Jun)	2.1	*29
Tumor suppresser	gb: NM_017061.1	Lysyl oxidase (Lox)	–2.3	*30
	gb: BI304009	Lysyl oxidase (Lox)	–1.5	*31
Extracellular matrix	gb: NM_031050.1	Lumican (Lum)	1.6	
	gb: NM_019164.1	Chondroadherin (Chad)	2.4	
	gb: NM_017087.1	Biglycan (Bgn)	–1.5	*32
	gb: U65656.1	Matrix metalloproteinase 2 (72 KDa type IV collagenase) (Mmp2)	–1.6	*33
	gb: NM_022221.1	Neutrophil collagenease (Mmp8)	–2.0	*34
	gb: AF034218.1	Hyaluronidase 2 (Hyal2)	–1.8	*35
	gb: Z78279.1	Collagen, type 1, alpha 1 (Col1a1)	–2.1	*36
	gb: BI285575	Collagen, type 1, alpha 1 (Col1a1)	–2.1	*37
	gb: BM388837	Procollagen, type I, alpha 2 (Col1a2)	–1.7	*38
	gb: AF305418.1	Procollagen, type II, alpha 1 (Col2a1)	1.7	*39
	gb: BI275716	Collagen, type III, alpha 1 (Col3a1)	–1.6	*40
	gb: AI230238	Procollagen, type X, alpha 1 (Col10a1)	2.9	b
	gb: AI235948	Nidogen (entactin)(Nid)	–1.5	*41
	gb: J04035.1	Elastin (Eln)	–2.5	*42
	gb: NM_012656.1	Secreted acidic cysteine rich glycoprotein (Sparc)	–1.7	*43
	gb: D28875.1	Secreted acidic cysteine rich glycoprotein (Sparc)	–1.6	*44
Cell recognition	gb: AA945737	Chemokine receptor (LCR1) (Cxcr4)	1.6	
	gb: U54791.1	Chemokine receptor (LCR1) (Cxcr4)	1.8	
	gb: NM_012513.1	Brain derived neurotrophic factor	1.5	
Cell-cell signaling	gb: NM_053822.1	S100 calcium-binding protein A8 (calgranulin A) (S100a8)	–2.0	
	gb: NM_053587.1	S100 calcium-binding protein A9 (calgranulin B) (S100a9)	–1.5	
	gb: NM_021654.1	Gap junction membrane channel protein alpha 4 (Gja4)	–2.0	
Cell adhesion	gb: NM_012976.1	Lectin, galactose binding, soluble 5 (Lgals5)	–1.5	
Cell growth	gb: AA944827	Bone morphogenetic protein 2 (Bmp2)	–1.6	
Cell proliferation	gb: AI008792	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (Id2)	2.0	
Cell cycle	gb: NM_133572.1	Cell division cycle 25B (Cdc25b)	–2.2	
Sodium channel	gb: AA685184	Sodium channel beta 3 subunit (Scnb3)	–1.7	

(*Continued*)

Classification	Accession no.	Gene name	FC	
Hormone	gb: NM_012703.1	Thyroid hormone responsive protein (Thrsp)	4.4	b
	gb: AI169092	Thyroid hormone responsive protein (Thrsp)	3.3	b
	gb: NM_053469.1	Hepcidin antimicrobial peptide (Hamp)	1.9	
	gb: NM_012612.1	Natriuretic peptide precursor type A (Nppa)	3.4	
Muscle	gb: AI711147	Actin alpha cardiac 1 (Actc1)	1.6	b
	gb: NM_019131.1	Tropomyosin 1, alpha (Tpm1)	2.6	b
	gb: AF372216.1	Tropomyosin 1, alpha (Tpm1)	1.6	
	gb: AF370889.1	Tropomyosin 1, alpha (Tpm1)	3.1	b
	gb: NM_017144.1	Troponin 1, type 3 (Tnni3)	5.5	b
	gb: NM_012676.1	Troponin T2 (Tnnt2)	3.5	b
	gb: NM_017240.1	Myosin heavy chain, polypeptide 7 (Myh7)	2.8	
	gb: NM_017239.1	Myosin heavy chain, polypeptide 6 (Myh6)	4.4	b
	gb: NM_057144.1	Cysteine-rich protein 3 (Csrp3)	1.8	b
Calcium ion binding	gb: AW520914	Calsequestrin 2 (Casq2)	1.9	
	gb: NM_017290.1	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (ATP2a2)	1.6	
	gb: NM_017309.1	Protein phospatase 3, regulatory subunit B, alpha isoform, type 1(Ppp3r1)	2.9	a
	gb: BI290034	Phospholamban (Plm)	2.1	
	gb: AI231802	Phospholamban (Plm)	1.7	
	gb: BF283732	Annexin III (Lipocortin III) (Anx3)	−1.6	
Signal transduction	gb: BE112453	Enigma homolog (Enh)	2.0	
	gb: NM_031530.1	Small inducible cytokine A2 (Scya2)	1.9	
	gb: NM_053826.1	Pyruvate dehydrogenase kinase 1 (Pdk1)	1.7	
	gb: NM_012719.1	Somatostatin receptor 1 (Sstr1)	1.7	
	gb: NM_012719.1	ADP-ribosylation factor 3 (Arf3)	2.1	
	gb: AI598401	Proviral integration site 1 (Pim1)	2.1	a
	gb: BG668493	Stathmin-like 2 (Stmn2)	−1.7	
	gb: NM_133380.1	Interleukin 4 receptor (Il4r)	−1.9	
	gb: AF411318.1	Metallothionein (Mtla)	−1.6	
	gb: BE101336	Kruppel-like factor 9 (Klf9)	−1.7	
Transport	gb: NM_134326.1	Albumin (Alb)	3.4	a
	gb: NM_024162.1	Fatty acid binding protein 3 (Fabp3)	2.5	b
	gb: NM_053365.1	Fatty acid binding protein 4 (Fabp4)	3.7	b
	gb: NM_053910.1	Pleckstrin homology, Sec7 and coiled/coil domains 1 (Pscd1)	1.9	
	gb: AB039825.1	Alpha-2u globulin PGCL4 (OBP3)	2.0	
	gb: NM_019157.1	Aquaporin 7 (Aqp7)	1.8	
	gb: NM_021588.1	Myoglobin (Mb)	3.1	
	gb: NM_012751.1	Solute carrier family 2, member 4 (Slc2a4)	2.4	
	gb: NM_031818.1	Chloride intracellular channel 4 (Clic4)	1.7	
	gb: BE113640	Solute carrier family 4, member 1 (Slc4a1)	−1.7	
	gb: AA891661	Aquaporin 1 (Aqp1)	−1.5	
	gb: NM_031703.1	Aquaporin 3 (Aqp3)	−2.1	
Phase 1 reactions enzyme	gb: NM_012542.1	Cytochrome P450, subfamily IIA (phenobarbital-inducble)/ (Cytochrome P450 IIA3) (CYP2A3a)	1.8	b
Electron transport	gb: NM_012812.1	Cytochrome c oxidase, subunit VIa, polypeptide 2 (Cox6a2)	3.2	b
	gb: NM_012682.1	Uncoupling protein 1 (Ucp1)	5.6	
	gb: NM_019354.1	Uncoupling protein 2 (Ucp2)	−1.9	
	gb: NM_031010.1	Arachidonate 12-lipoxygenase (Alox12)	−1.7	
Regulation of transcription	gb: AW672589	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha (Nfkbia)	−1.9	*15
	gb: NM_012551.1	Early growth response 1 (Egr1)	2.3	
	gb: NM_019194.1	Thyrotroph embryonic factor (Tef)	2.9	
	gb: AF286470.2	Sterol regulatory element binding factor 1 (Srebf1)	1.7	*11
	gb: AI230048	D site albumin promoter binding protein (Dbp)	2.0	
	gb: NM_031678.1	Period homolog 2 (Per2)	1.9	
	gb: NM_012780.1	Aryl hydrocarbon receptor nuclear translocator (Arnt)	1.6	
	gb: BF419200	CCAAT/enhancerbinding, protein (C/EBP) delta (Cebpd)	−2.1	*12
	gb: NM_031786.1	Tripartite motif protein 3 (Trim3)	−1.6	
Translation	gb: BE107346	Eukaryotic initiation factor 5 (eIF-5) (Eif5)	1.5	
Post translational modification	gb: AF062741.1	Pyruvate dehydrogenase phosphatase isoenzyme 2 (Pdp2)	1.6	
	gb: M18769.1	Sialyltransferase 1 (Siat1)	1.5	
	gb: BI300565	A disintegrin and metalloprotease domain 10 (Adam10)	1.6	

(*Continued*)

Classification	Accession no.	Gene name	FC
Metabolism	gb: AA848319	Lactate dehydrogenase B (Ldhb)	1.7
	gb: NM_012530.1	Creatine kinase, muscle (Ckm)	2.0
	gb: NM_057125.1	Peroxisomal biogenesis factor 6 (Pex6)	2.1
	gb: NM_080886.1	Sterol-C4-methyl oxidase-like (Sc4mol)	1.5
	gb: NM_013089.1	Glycogen synthase 2 (Gys2)	5.3
	gb: NM_021664.1	DNaseII-like acid DNase (Dlad)	1.7
	gb: U08027.1	Glycerol-3-phosphate dehydrogenase 2	1.5
	gb: NM_022215.1	Glycerol 3-phosphate dehydrogenase (Gpd3)	3.2
	gb: U36771.2	Glycerol-3-phosphate acyltransferase, mitochondrial (Gpam)	1.5
	gb: D87247.1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (Pfkfb3)	2.0
	gb: BI295900	Dihydrolipoamide acetyltransferase (Dlat)	2.0
	gb: AA850780	trehalase (Treh)	1.7
	gb: NM_013197.1	Aminolevulinic acid synthase 2 (Alas2)	–2.1
	gb: NM_031315.1	Cytosolic acyl-CoA thioesterase 1 (Cte1)	–2.3
	gb: Y00714.1	Alkaline phosphatase, tissue-nonspecific (Alp1)	–1.6
	gb: AI717733	Paraoxonase 1 (Pon1)	–1.8
	gb: NM_022592.1	transketolase(Tkt)	1.6
Regulation of ossification	gb: BE107528	Smad5 (Madh5)	1.9
	gb: AI409634	Best5 protein (Best5)	–1.8
Cholesterol Biosynthesis	gb: NM_031840.1	Farensyl diphosphate synthase (Fdps)	1.5
	gb: M33648.1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgcs2)	–3.3
Development	gb: NM_053601.1	Neuronatin (Nnat)	–1.8
Other	gb: Z18877.1	25 oligoadenylate synthetase (Osa1)	–1.5

Note: FC: fold of change (SO_2 exposure group/control)
*1-46: the genes those are described in Results and Discussion.
a) the genes which their expressions in both short- and long-term groups were changed in the same direction(up- or down-regulated).
b) the genes which their expressions in both short- and long-term groups were changed in different direction (up- and down-regulated).

enzymatic activities (Salmon et al., 2004). These results imply that Ca3 and Prdx6 are expressed as an adaptive response to oxidative stress caused by lower SO_2 concentrations. The inhibitory effects of SO_2 on expression of Cp may indicate an inability of the Cp to adapt effectively.

3.2.3 Oncogene and tumor suppresser

It is reported that c-jun mRNA was expressed in alveolar macrophages from rats exposed to air containing 100 mg/m^3 volcanic ash with or without 1.5 ppm SO_2 (Samukawa et al., 2003). Similar results were seen in the present study. Jun was up-regulated as well as c-fos oncogene (c-fos) and v-ets erythroblastosis virus E26 oncogene like (avian)(Erg) (Table 2-*27-29). Meanwhile, lysyl oxidase (Lox), which is a tumor suppressor gene inactivated by methylation and loss of heterozygosity in gastric cancers, was down-regulated (Table 2-*30-31).

The overexpression of oncogene, combined with suppression of tumor suppressor gene, may result in uncontrolled tumor growth by SO_2 inhalation.

3.2.4 Extracellular matrix (ECM)

In the present study, we found that many major macromolecules of ECM were down-regulated in lungs of rats long-term exposed to SO_2 (Table 2-*32-34). Oxidant products may cause activation of precursor forms of collagenase or gelatinase, leading to breakdown of the extracellular matrix (McCauley and Matrisan, 2001). Various studies have demonstrated that cellular functions and responses can be affected by the surrounding ECM, and it is also well established that ECM plays a crucial role in maintaining the structural as well as functional integrity of cells (Sage and Bornstein, 1991; Yao and Eghbali, 1992; Sawhney et al., 1997). Keeping all these facts in mind, it becomes clear that any alterations in the balanced production and breakdown of ECM components could result in a pathologic condition (Sporn et al., 1986). Secreted acidic cysteine rich glycoprotein (SPARC) comprises a group of calcium-binding matricellular glycoproteins that are secreted by many different types of cells (Table 2-*43-44). They exhibit counter-adhesive effects that lead to cell rounding and changes in cell shape that result in the disruption of cell-matrix interactions. It has been shown that SPARC interacts with various molecules including cations (Ca^{2+}, Cu^{2+} and F^{2+}), growth factors, ECM proteins (collagens, bitronectin), and many others (Yan and Sage, 1999).

It showed that SO_2 or its derivatives might regulate cell–substrate interactions, affect the cell adhesion, spreading and signaling, and hence regulate a wide variety of biological functions, including cell growth, cell migration, cell differentiation, synthesis of extracellular matrix and tissue morphogenesis.

3.3 Changed genes in short-term and long-term exposed to SO_2

Interestingly, we found only three genes were commonly up-regulated in both of treatment groups (Table 1, 2-a). However, two of thirty-one genes up-regulated in short-term group were down-regulated in long-term group and sixteen of thirty genes down-regulated in short-term group were up-regulated in long-term group (Table 1, 2-b).

Fatty acid binding protein (Fabps), THRP and muscle components were involved in those genes changed in different direction. Fabps are small cytosolic proteins with virtually identical backbone structures that facilitate the solubility and intracellular transport of fatty acids.

These findings cannot be explained, but it is believed that there were different, or even distinct mechanisms at low-dose long-term and high-dose short-term exposed to SO_2.

4 Conclusions

We presented in the current study that 62 genes that were up- or down-regulated in lungs of rats exposed to short-term SO_2 and 261 genes that were up- or down-regulated in lungs of rats exposed to long-term SO_2. The expression of few genes changed by more than 50% in the same direction in lungs of rats exposed to short-term and long-term SO_2, which indicates that SO_2 exerts its effects by different mechanisms in vivo at high-dose short-term inhalation and at low-dose long-term inhalation. A notable feature of the gene expression profile was the decreased expression of genes related to oxidative phosphorylation in lungs of rats exposed to short-term SO_2, which shows that high-dose short-term exposed to SO_2 may cause the deterioration of mitochondrial functions. Discriminating genes in lungs of rats exposed to long-term SO_2 included those involved in fatty acid metabolism, immune, inflammatory, oxidative stress, oncogene, tumor suppresser and extra cellular matrix. The mechanism of low-dose long-term exposed to SO_2 is more complex.

This study is very important for understanding the key genes participating in possible mechanism of SO_2 toxicological damages and biological roles. There were many other genes that seemed to be important for pulmonary function in animals exposed to SO_2, though remain to be studied in the future. Thus, we are also performing more exhaustive analysis.

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