Proteomic Identification of Macrophage Migration-inhibitory Factor upon Exposure to TiO2 Particles*

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Inhalation of particulate matter aggravates respiratory symptoms in patients with chronic airway diseases, but the mechanisms underlying this response remain poorly understood. We used a proteomics approach to examine this phenomenon. Treatment of epithelial cells with BSACoated titanium dioxide (TiO2) particles altered 20 protein spots on the two-dimensional gel, and these were then analyzed by nano-LC-MS/MS. These proteins included defense-related, cell-activating, and cytoskeletal proteins implicated in the response to oxidative stress. The proteins were classified into four groups according to the time course of their expression patterns. For validation, RT-PCR was performed on extracts of in vitro TiO2-treated cells, and lung issues from TiO2-treated rats were analyzed by immunohistochemical staining and enzyme immunoassay. TiO2 treatment was found to increase the amount of mRNA for macrophage migration-inhibitory factor (MIF). MIF was expressed primarily in epithelium and was elevated in lung tissues and bronchoalveolar lavage fluids of TiO2-treated rats as compared with sham-treated rats. Carbon black and diesel exhaust particles also induced expression of MIF protein in the epithelial cells. Molecular & Cellular Proteomics 6:56–63, 2007.

Increasing epidemiological evidence indicates that inhalation of airborne particulate matter (PM) is associated with adverse health outcomes, such as respiratory and cardiac mortality and morbidity (1, 2). The lung function of patients with chronic obstructive pulmonary disease declines more quickly in those patients who live in communities exposed to high levels of air pollution compared with those patients living in areas with low pollution (3). Levels of environmental particles also positively correlate with exacerbation of asthma (4). During the last decade, the composition of air pollution in developed countries has changed from classical type 1, consisting of SO2 and large dust particles, to modern type II, which is characterized by oxides of nitrogen, organic compounds, ozone, and ultrafine particles (5).

Airborne particulate matter with an aerodynamic diameter of less than 10 µm (PM10) is a complex mixture of organic and inorganic compounds containing sulfates and various metals such as aluminum, calcium, copper, iron, lead, magnesium, titanium, and zinc (6). Clinically PM10 particles are thought to provoke airway inflammation by stimulating the release of mediators that exacerbate lung disease in susceptible individuals (4, 7). This hypothesis is based on experimental evidence that direct instillation or inhalation of PM10 particles is followed by airway inflammation in animal models (8).

TiO2 particles are a PM10 component found in dusty workplaces in industries involved in the crushing and grinding of the mineral ore rutile (9). Fifty percent of TiO2-exposed workers have respiratory symptoms accompanied by reduction in pulmonary function (10). Because acute and chronic exposures to TiO2 particles also induce inflammatory responses in the airways and alveolar spaces of rats (11–14), TiO2-treated rats are a good model for the study of the human epithelial response to PM10 particles.

These fine and ultrafine particles directly stimulate macrophages and epithelial cells to produce both inflammatory cytokines, such as tumor necrosis factor-α, transforming growth factor-β1, granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, IL-6, and IL-8, and reactive oxygen species (ROS) (15–21). We previously reported that particle exposure causes the antigen-sensitized lung to favor a Th2 environment with overproduction of IL-13 and IL-25 (11, 12). However, a comprehensive examination of the mediators produced by particle-stimulated epithelial cells has not been performed.

Proteomics offers a unique means of analyzing the expressed genome, and it has been successfully used to examine the generation of oxidative stress at the cellular level (22). In addition to revealing protein modifications, this approach can also be used to look at changes in protein expression levels (18). In this study, we adopted a proteomics approach...
to identify the protein changes that occur in epithelial cells in response to exposure to BSA-coated TiO₂ particles. Two-dimensional electrophoresis data were validated by RT-PCR, and then the data were also proved using an animal model.

**MATERIALS AND METHODS**

**Cell Culture and Stimulation with TiO₂ Particles—**A human bronchial epithelial cell line (BEAS-2B) was obtained from the American Type Culture Collection (ATCC), Manassas, VA. The cells were maintained in Dulbecco’s modified Eagle’s medium/F-12 Nutrient Mixture Ham containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C in a 5% CO₂ incubator. For experimental treatment, the BEAS-2B cells were seeded into T-75 tissue culture flasks (2 × 10⁴ cells/ml) and cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C under 5% CO₂. One day after seeding, the cells were treated with various concentrations of BSA-coated TiO₂ particles and then cultured at 37 °C in a humidified CO₂ incubator. Fine rutile TiO₂ particles (mean diameter = 0.29 μm) were prepared as described previously (12).

**Two-dimensional (2-D) Electrophoresis and Image Analysis—**BEAS-2B cells were harvested by centrifugation and then disrupted with lysis buffer containing 5 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1% Triton X-100, and 2 mM PMSF. The cell lysate was centrifuged at 12,000 × g for 30 min, and the supernatant fraction was collected. Protein concentrations were determined using a BCA assay kit (Pierce). Immobilize DryStrips (Amersham Biosciences) was used for isoelectric focusing, which was carried out with 1 mg of the extracted protein on an IPGphor system (Amersham Biosciences). After IEF separation, the proteins were separated in the second dimension by SDS-PAGE.

For image analysis, the gels were visualized with Coomassie Brilliant Blue G-250 according to the manufacturer’s instructions. The 2-D gels were scanned with an ImageScanner (Amersham Biosciences) in transmission mode. Spot detection and matching were performed using ImageMaster 2D version 5.0 (Amersham Biosciences). Digitized images were analyzed using the ImageMaster program to calculate the 2-D spot intensity by integrating the optical density over the spot area (the spot “volume”) and normalized. The values were normalized and then exported to SPSS 8.0 for statistical analysis.

**Protein Identification by Nano-LC-MS/MS and Database Searching—**Differentially expressed protein spots (see “Statistical Analysis for details”) were excised from the 2-D gels, cut into smaller pieces, and digested with trypsin (Promega) as described previously (23). All LC-MS/MS experiments were performed using an Agilent Nanoflow Proteomics Solution featuring an Agilent 1100 Series nano-LC system for MS/MS coupled through an orthogonal nanospray ion source to an Agilent 1100 Series LC/MSD Trap XCT ion trap mass spectrometer. The nano-LC system was operated in sample enrichment/desalt mode with a ZORBAX 300SB-C18 enrichment column (0.3 × 50 mm, 5 μm). Chromatography was performed using a ZORBAX 300SB-C18 (75-μm × 150-mm) nanocolumn. The column was eluted with a gradient beginning with isocratic application of 3% solvent B (0.1% formic acid in acetonitrile) and 97% solvent A (0.1% formic acid in water) for 5 min. The gradient mixture was then changed to 10% B over 5 min (from 5 to 10 min), to 45% B over 40 min (10–50 min), to 90% B (isocratic) for 5 min (55–60 min), and to 3% B over 1 min (60–61 min), and finally the column was washed with 3% B for 10 min.

The LC/MSD Trap XCT was operated in the unique peptide scan auto-MS/MS mode. The ionization mode was positive nanoelectrospray with an Agilent orthogonal source. Drying gas flowed at 5 liters/min at a temperature of 300 °C. Vcap was typically 1800–1900 V with skim 1 at 30 V, and the capillary exit was offset at 75 V. The trap drive was set at 85 V with averages of one or two. Ion charge control was on with maximum accumulation time of 150 ms, the smart target was 125,000, and the MS scan range was 300–2200. Automatic MS/MS was performed in ultrasonic mode with the number of parents 2, averages of two, a fragmentation amplitude of 1.15 V, Smart Frag on (30–200%), active exclusion on (after two spectra for 1 min), prefer +2 on, MS/MS scan range of 100–1800, and ultrasonic on. Each acquired MS/MS spectrum was searched against the non-redundant protein sequence database using Spectrum Mill software.

**Semiquantitative RT-PCR—**Total RNA was isolated from 2 × 10⁷ cultured BEAS-2B cells using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. To eliminate genomic DNA from the samples, DNase I treatment (Qiagen) was included in the RNA isolation procedure. The cDNA was prepared using a Superscript II kit (Invitrogen) and used as a template for analysis of gene expression by PCR. Primers and probes were designed for the selected genes using GeneFisher software (53). The following primer sequences (shown 5’ → 3’) were used: glyceraldehyde-3-phosphate dehydrogenase (GAPDH); CTGCT TCACCC AACCT GGAGA (for) and CGGCC ATCAG GCCAC AGTTT (reverse); transaldolase (TALD01); CTACA AGGAA GCTGG GATC (forward) and CAACC AACGG AAAGA CTTC (reverse); chloride intracellular channel 1 (CLIC1); GGTTG CCATT AACCT GTAC (forward) and TAGGC ATTCG TCAAG TAC (reverse); macrophage migration-inhibitory factor (MIF); CGATC ATGCG GATGT TC (forward) and CGAAG GTGGA GTTGT TC (reverse). Gene expression was measured in six independent cultures of BEAS-2B cells, and all measurements were performed in duplicate.

**Western Blot Analysis of MIF Expression—**BEAS-2B cells were cultured in the presence of 20 μg/ml TiO₂ carbon black, or diesel exhaust particles for 8 or 48 h. Crude cellular extracts were obtained as described above for 2-D electrophoresis, and Western blot analysis was performed according to the method of Towbin et al. (24). Proteins were fractionated by 15% SDS-PAGE and transferred to a nitrocellulose membrane (Amersham Biosciences). The membrane was blocked for 1 h in PBS containing 1% BSA and then incubated with polyclonal rat anti-human MIF antibody (1:2000 dilution) for 2 h at room temperature. The unbound primary antibodies were removed with three 10-min washes in PBS containing 0.01% (v/v) Nonidet P-40. The membrane was then incubated with peroxidase-conjugated anti-rat IgG (Sigma) for 30 min. ECL detection of MIF was performed according to the manufacturer’s instructions (Roche Applied Science). Analysis of MIF Expression in Lung Tissue of TiO₂-instilled Rats by Immunohistochemical Staining and EIA—A TiO₂-treated rat model was prepared as described in our previous publications (11, 12) using male Sprague-Dawley rats, 7 weeks of age. Rats received 4 mg of TiO₂ in 0.2 ml of endotoxin-free water by intratracheal instillation or were sham-treated. Immunohistochemistry was performed to examine the secretion of MIF. Four-micrometer tissue section slides were treated with 0.3% H₂O₂-methanol for 30 min to block endogenous peroxidase and then incubated with biotinylated anti-goat MIF antibody (1:100 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 4 °C overnight. After washing with Tris-buffered saline the slides were incubated with avidin-biotin peroxidase complex (ABC kit, Vector Laboratory, Burlingame, CA). Color was developed by staining with 3,3’-diaminobenzidine tetrachloride (Zymed Laboratories Inc., San Francisco, CA).

The amount of MIF protein in bronchoalveolar lavage fluid of TiO₂- or sham-treated rats was determined by EIA. All EIA procedures were performed according to the manufacturer’s protocol (Chemicon International, Inc.). Inter- and intra-assay coefficients of variance were less than 10%.

**Statistical Analysis—**Statistical analysis was performed using
SPSS 8.0 software. Differences in spot intensity on 2-D gels were compared between three independent groups or samples using the non-parametric Kruskal-Wallis H test for continuous data. If differences were found significant, the Mann-Whitney U test (two-sample rank sum test) was applied to the differences in densities and MIF concentrations of the two groups. All data were expressed as median values (interquartile range), and significance was defined as p < 0.05.

**RESULTS**

2-D Electrophoresis and Protein Identification—A proteomics approach was used to determine the differential expression of proteins at 8 and 48 h after treatment of a BEAS-2B cell line with 20 μg/ml BSA-coated TiO2 particles. Cytosolic fractions were obtained by differential centrifugation and then separated by 2-D electrophoresis in six replicate gels per treatment. A representative image of the proteomic profile of BEAS-2B cells prior to treatment is shown in Fig. 1. A total of 650 (median, 652; range, 635–693) protein spots were detected on each gel. All of the identified spots were localized in the pI 3–10 range with a molecular mass range of 10–150 kDa. This 2-D gel image was used as a master gel and reference map.

2-D PAGE of extracts from TiO2-treated cells revealed 20 spots that changed by more than 2-fold at 8 or 48 h after treatment. These spots were excised from the gel and incubated with trypsin to digest the proteins in the gel, which were then analyzed by LC-MS/MS. The results of this analysis are summarized in Table I.

Cluster Analysis—The expression profiles of the 20 proteins with significant (p < 0.05) differential expression were visualized using a hierarchical clustering algorithm (dCHIP software).
Four fundamental profile patterns could be identified from the clusters: continuously increasing (Fig. 2A, group 1), transiently increasing (Fig. 2B, group 2), continuously decreasing (Fig. 2C, group 3), and transiently decreasing (Fig. 2D, group 4). The proteins in each group are summarized in Table I. The H⁺-transporting ATPase (ATP6V1B2), keratin 6A (K6A), and MIF were included in group 1. The 60-kDa heat-shock protein 1 (HSPD1), the RUVBL2 ATPase/helicase, proliferating cell nuclear antigen (PCNA), TALDO1, and CLIC1 were in group 2. A continuous decrease in MCM7, calpain 1 (CAPN1), ribonuclease/angiogenin inhibitor (RNH1), vimentin (VIM), 26 S proteasome subunit 9 (PSMD9), and actin-related protein was observed in group 3. A transient decrease in PRP19/PSO4 pre-mRNA processing factor 19 homologue (PRP19), ribosomal protein P0 (RPLP0), 27-kDa heat-shock protein 1 (HSPB1), phosphoglycerate mutase 1 (PGAM1), and platelet-activating factor acetylhydrolase, isoform lb (PAFAH1B3) was observed in group 4.

Expression of MIF mRNA Is Increased by Treatment with TiO₂ Particles—The mRNA expression levels of one up-regulated protein (MIF) and two transiently induced proteins (TALDO1 and CLIC1) were evaluated by semiquantitative RT-PCR. As shown in Fig. 3, MIF mRNA expression was increased at 8 and 48 h after treatment with TiO₂ particles. After normalization against GAPDH mRNA expression, the data for TALDO1 and CLIC1 mRNAs did not reveal a significant change.

Identification of MIF-expressing Epithelial Cells in TiO₂-treated Rat Lung—To determine whether MIF expression in lung tissue is elevated after in vivo stimulation with TiO₂ particles, we instilled TiO₂ particles in rats as described previously (11). Expression of MIF protein in intrapulmonary bronchi was examined by immunohistochemical staining. Forty-eight hours after instillation of saline, no MIF protein was detected (Fig. 4A, left), but 48 h after instillation of TiO₂ particles, MIF was detected in the epithelial cells of the bronchi and bronchioles (Fig. 4A, right).

Western blot analysis of homogenates from sham- and TiO₂-treated rat lung lysates (100 μg/lane) showed that MIF protein significantly increased in lung from TiO₂-treated rats compared with that from untreated rats (Fig. 4B). When MIF concentrations were measured in bronchoalveolar lavage fluid of sham- and TiO₂-treated rats using ELISA, they were found to be significantly higher in the latter than in the former (Fig. 5).

Production of MIF Protein by BEAS-2B Cells Stimulated with Different Types of Particles—To ascertain the specificity of the response of epithelial cells to TiO₂ particles, BEAS-2B cells were treated with 20 μg/ml TiO₂, carbon black, or diesel exhaust particles as stimulants. In Western blots of the cell lysates, the MIF signal intensity increased at 8 and 48 h after stimulation of TiO₂, and a similar pattern of change was observed. 

![Fig. 2. Cluster analysis of 20 proteins with significant differential expression (>2-fold change) at 8 or 48 h caused by TiO₂ treatment of BEAS-2B epithelial cells. The expression profiles of the individual proteins were classified by cluster analysis. Protein names (National Center for Biotechnology Information (NCBI)) are displayed for each cluster.](image)

![Fig. 3. Semiquantitative RT-PCR analysis of TALDO1, CLIC1, and MIF mRNA expression. The levels of mRNA for TALDO1, CLIC1, and MIF were estimated in extracts of BEAS-2B cells stimulated with TiO₂ particles (20 μg/10⁶ cells). Expression levels were determined by densitometry and normalized to the level of GAPDH mRNA.](image)
observed after stimulation with carbon black or diesel exhaust particles (Fig. 6).

**DISCUSSION**

Although air pollution containing heavy metallic environmental particles increases morbidity and mortality of patients with chronic airway diseases (3, 4), the details of the mechanisms underlying particulate-induced airway obstruction are not understood. Epithelial cells, which are the first type of cells to be exposed to inhaled particles, respond to particulate exposure by producing several cytokines, chemokines, and small molecule mediators, leading to airway inflammation and other physiological changes, (9–11). However, a clear picture of the overall pattern of the epithelial cell response to particulates is lacking. To better understand the molecular pathogenesis of this response, we adopted a proteomics approach. Using 2-D electrophoresis as a screening tool, we were able to identify several proteins that exhibited differential expression in untreated and TiO₂-treated BEAS-2B cells in vitro, and we then validated this result at the transcriptional level and in an in vivo model. We believe that this report is the first to use proteomics to develop a single map showing the proteins differentially expressed in the cytoplasm of epithelial cells after stimulation with particulate matter.

Our previously published (12) and unpublished² flow cytometry data showed that the value of side scatter indicating endocytosis is maximal at 8 h after TiO₂ treatment and returns to base-line levels within 48 h. The optimal concentration of TiO₂ for this effect was 20 μg/ml per 10⁵ cells (data not shown). Apoptosis of bronchial epithelial cells occurs at concentrations of at least 10 μg/ml (25). Therefore, we treated 10⁵ BEAS-2B cells for 8 and 48 h with 20 μg/ml TiO₂, diesel exhaust, or carbon black particles.

One of the factors that determines the impact of particulates on epithelial cells is oxidative stress (8, 9, 12, 13). Cellular homeostasis depends on the balance between ongoing generation of ROS and antioxidant defenses. When ROS production overwhelms the antioxidant defense system, oxidative stress occurs (26). Oxidative stress can elicit a range of cellular responses with effects ranging from protective to injurious; the more damaging responses include initiation of inflammation and activation of programmed cell death. Particulate exposure is more likely to be cytotoxic to bronchial epithelial cells than to macrophages (25). Interestingly the 20

² M.-H. Cha, T. Rhim, K. H. Kim, A.-S. Jang, Y.-K. Paik, and C.-S. Park, unpublished data.
proteins that we identified as affected by TiO₂ particle exposure could reasonably be grouped into three categories based on their known functions: defense-related proteins, cell-activating proteins, and cytoskeletal proteins, all of which could be linked to the response to oxidative stress.

TALDO1, an essential enzyme of the pentose phosphate pathway, generates reducing equivalents to protect cellular integrity from damage due to reactive oxygen intermediates (27). The effect of TiO₂ treatment on the expression profile of PGAM1, an enzyme of the glycolysis pathway, was functionally opposite to its effect on that of TALDO1 protein. Eight hours after treatment, TALDO1 expression increased, whereas PGAM1 expression decreased, and 48 h after treatment, the former decreased, and the latter increased. These data suggest that epithelial cells utilize the machinery of gluconeogenesis to protect against the harmful effects of particulates soon after exposure and that the actions of the glycolysis pathway overwhelm gluconeogenesis at later times after exposure.

ATP6V1B2, a component of vacuolar ATPase, mediates acidification of eukaryotic intracellular organelles that are necessary for such intracellular processes as protein sorting, zymogen activation, receptor-mediated endocytosis, and generation of a proton gradient by synaptic vesicles (28). Levels of ATP6V1B2 persistently increased in our study, indicating that at least some of these intracellular processes were actively ongoing.

Levels of the defense-related heat-shock proteins HSPD1 and HSPB1 (29, 30) increased at 8 and 48 h after exposure, respectively. This result may be an indication of enhanced defense against oxidative stress via several different heat-shock proteins. Oxidized protein derivatives generated as a result of oxidative stress tend to aggregate, and accumulation of these aggregates may lead to cell death (31). To prevent this occurrence, the oxidized proteins, as well as modified nucleotides, are selectively recognized and either repaired or degraded by the proteosome pathway (31). Levels of 26 S proteosome subunit 9 (PSMD9) and 26 S proteosome-associated pad1 homologue (32) continuously decreased after stimulation of cells with TiO₂ particles, suggesting that certain defense mechanisms may break down after particulate exposure.

CAPN1, VIM, and actin-related protein 2 (ACTR2) are essential for cell structure and motility, playing critical roles in cell migration and differentiation (33–35); they also defend cells against oxidative stress (36). The time-dependent decrease of these proteins suggests that epithelial cells lose their intracellular supporting structures after stimulation with TiO₂. PGNA, CLIC1, and K6A are induced in response to stressful stimuli such as wounding (37). Because a TiO₂ stimulus can induce phenotype changes of goblet or epithelial cells (11), the TiO₂-induced increase in these proteins may be understood as part of an overall change in phenotype. The RNH1 and RPLP0 proteins are essential for control of mRNA turnover (38), and the RUVBL2 and MCM7 proteins respond to stress and participate in repair of DNA damage (39, 40). Thus, the observed changes in levels of these proteins after particulate exposure may indicate that the exposure disrupts transcription, translation, and cellular activation.

Based on our proteomic data, we speculate that the proteolysis of oxidized proteins by, or antioxidant activity of, the TALDO1, ATP6V1B2, HSPD1, HSPB1, PSMD9, and POH1 proteins is part of the initial defense against oxidative stress. If these antioxidant and detoxification mechanisms fail to reduce oxidative stress, more damaging responses, including initiation of inflammation and activation of programmed cell death, will occur. Furthermore particle exposure may alter expression of the RNH1, RPLP0, RUVBL2, and MCM7 genes, thus affecting transcription, translation, and cellular activation as part of a healing or apoptotic process. Consequently the epithelial cells may begin to express genes related to phenotype change, such as those for PCNA, CLIC1, and K6A. As all of these processes are taking place, the epithelial cells synthesize inflammatory mediators such as PAFAH1B3 and MIF (41, 42). We have not evaluated whether the alterations in expression of these proteins are specific to particulate exposure. To our knowledge, the present proteomics study provides the first set of advanced and integrated data concerning the response of epithelium to particulates.

Of the 20 proteins we identified, we selected three, TALDO1, MIF, and CLIC1, for closer examination because these proteins are involved in metabolism and inflammation (27, 42, 43). To determine whether the effect of TiO₂ particles on expression of these proteins occurs at the level of transcription or translation, we performed semiquantitative RT-PCR to estimate mRNA levels. We found that levels of MIF mRNA were elevated by about 3–7-fold in TiO₂-treated cells as compared with untreated cells (Fig. 3). This result agrees with our results from immunoblotting (Fig. 5) and 2-D electrophoresis. In contrast, mRNA levels for TALDO1 and CLIC1 did not vary with TiO₂ treatment (Fig. 4), suggesting that the observed alterations in levels of the TALDO1 and CLIC1 proteins were caused by post-transcriptional changes in stability of TALDO1 and CLIC1 mRNA and/or protein.

Because MIF was reported to be a proinflammatory cytokine that counteracts the anti-inflammatory effect of endogenous glucocorticoids (42, 44), its role in human and experimental asthmatics has been demonstrated. MIF levels in the serum and sputum of human asthmatics were shown to significantly higher than those of age- and sex-matched control subjects (45, 46). In addition, in a genomics study of MIF, both the −173G/C and −794(CATT)₈ repeat polymorphisms in the MIF promoter region were associated with altered levels of MIF gene transcription in vitro. Furthermore a case-controlled analysis demonstrated that these promoter polymorphisms exerted a genetic influence on the development of atopy and asthma in a Japanese population (47). Evidence that MIF plays a role in asthma development also has been obtained
using ovalbumin-challenged allergic asthma models. Mice with an MIF deficiency caused by either genetic alteration or a neutralizing antibody exhibited less pulmonary inflammation, a lower Th2 immune response, and lower airway hyperresponsiveness than did those of genetically matched, wild-type controls (48, 49).

MIF was once thought to be expressed primarily in peripheral blood and airway eosinophils (45, 46). However, a recent immunohistochemical study revealed positive immunostaining for MIF in bronchial epithelium, even in non-sensitized rats, with enhanced MIF staining occurring after ovalbumin challenge (49). These data are in good agreement with ours in that they indicate that epithelial cells also express MIF. Interestingly MIF has been shown to regulate innate immune responses to endotoxin (50, 51) partly via modulation of toll-like receptor 4 expression (52).

The immunopathogenesis of the particulate model (innate) of airway inflammation has been considered to be quite different from that of the ovalbumin-challenged model (adaptive). To our knowledge, our results provide the first clear demonstration that MIF production in epithelial cells is induced by various kinds of particulates, including carbon black and diesel exhaust particles (Fig. 6). Our MIF data and that from ovalbumin-challenged models (49) suggests that MIF plays an important role as a common inducer of airway inflammation regardless of the type of airway injury.

In summary, we identified 20 proteins whose expression levels in the BEAS-2B cell line changed in response to TiO2 particle exposure. These proteins include defense-related, cell-activating, and cytoskeletal proteins implicated in the response to oxidative stress, and they can be classified into four groups according to the pattern of their TiO2-induced change in expression over time. One of these proteins, MIF, was induced at the transcriptional level by stimulation of cells with any one of three different particulate molecules, and expression of MIF protein was increased in the lungs of TiO2-instilled rats. These results indicate that some of these proteins may serve as mediators of, or markers for, airway disease caused by exposure to PM.

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