Original Article

Protein Expression in Silica Dust-induced Transdifferentiated Rats Lung Fibroblasts*

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Abstract

Objective To analyze the expression of different proteins in free silica-induced transdifferentiated rat lung fibroblasts.

Methods Rat lung fibroblasts and alveolar macrophages were cultured. A transdifferentiation model of rat lung fibroblasts was established. Free silica was used as a stimulator for rat lung fibroblasts. Changes in α-SMA were detected by immunohistochemistry and Western blot, respectively. Protein of lung fibroblasts was extracted and analyzed by two-dimensional electrophoresis (2-DE).

Results Six protein spots were identified by mass spectrometry, including glyceraldehyde 3-phosphate-dehydrogenase, peroxiredoxin 5, heterogeneous nuclear ribonucleoprotein A2, transgelin 2, keratin K6 and vimentin.

Conclusion Some proteins are changed in free silica-induced transdifferentiaed rat lung fibroblasts.

Key words: Transdifferentiation; Myofibroblast; Two-dimensional electrophoresis; Crystalline silica

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INTRODUCTION

Transdifferentiation takes place when non-stem cells are transformed into a different type of cells or when stem cells are differentiated outside their established differentiation path. Transdifferentiation is a type of metaplasia, which covers all cell fate switches, including interconversion of stem cells. At present, many cells are considered to be able to change their functions by transdifferentiation, such as lung fibroblasts.

It is known that lung fibroblasts play an important role in pulmonary fibrosis[4-6]. Pulmonary fibrosis can be activated directly or indirectly by exogenous factors, such as silica. In China, the hazard of silica to exposed workers is of a great concern[1-3]. Silicosis is a chronic pulmonary fibrosis disease due to inhaled silica-containing dusts. In the development of silicosis, macrophages can secrete a lot of cytokines, such as TNF-α, TGF-β_1, and PDGF[15-16]. These cytokines, especially TGF-β_1, induce normal lung fibroblasts to express α-smooth muscle actin (α-SMA), a typical cell marker of lung myofibroblasts[7-8], which secrete extracellular matrix (ECM) components such as collagens, and are mainly deposited in lungs[9-13]. Upon this occasion, the cells are transferred to lung myofibroblasts. Thus, transdifferentiation of lung fibroblasts is an important generative mechanism of myofibroblasts.

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In addition, myofibroblasts can be derived through activation and proliferation of resident lung fibroblasts[6], epithelial-mesenchymal differentiation[14], or recruitment of circulating fibroblastic stem cells (fibrocytes). However, the molecular mechanism of this process is not clear. Therefore, lung fibroblasts and macrophages were isolated from rats to observe their transdifferentiation mechanism in this study. Dioxide silica was employed to stimulate macrophages and collect the supernatant, which was used to treat lung fibroblasts. Proteins in lung fibroblasts and myofibroblasts were analyzed by two-dimensional electrophoresis (2-DE) in an attempt to find some key protein molecules and the possible mechanism.

MATERIALS AND METHODS

Chemicals and Reagents

DMEM was purchased from Solarbio Science and Technology (Beijing, China). Fetal bovine serum (FBS) was bought from Hangzhou Sijiqing (Hangzhou, China). SP immunohistochemistry kit was obtained from Zhongshan Limited (Beijing, China). Monoclonal mouse antibody against α-SMA was from BOSTER (Wuhan, China). Crystalline silica powder (99% particle size of 0.5-10 μm) was obtained from Occupational Health and Poison Control Institute of Chinese National CDC.

Immobiline DryStrips (pH 3-10, 24 cm) and immobilized pH gradient (IPG) buffer were bought from Bio-Rad. Dry strip cover fluids were from GE, and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), bromophenol blue, agarose, acrylamide, tris-base, glycine, sodium dodecyl sulfate (SDS), N,N,N',N'-tetramethylethylenediamine (TEMED), and coomassie brilliant blue R-250, dithiothreitol (DTT) and iodoacetamide were bought from Sigma. The remaining chemicals were of an analytical grade. All buffers were prepared with Milli-Q water.

Culture of Rat Lung Fibroblasts

Lung fibroblasts used for *in vitro* experiments were isolated from male SD rats (SPF, Henan Experimental Animal Center)[17]. Briefly, lung tissue was separated, minced and washed 3 times with D-Hank's buffer under sterile conditions, and disaggregated with crude bovine pancreatic trypsin. The cells were resuspended in DMEM containing 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. Approximate 1.0×10^6 cells were planted in each serum dilution bottle with a total volume of 10 mL media. The cells were trypsinized and replated after about 5 days of culture.

Culture of Rat Macrophages

Rat macrophages were collected from bronchoalveolar lavage (BAL) of SD rats. After the rats were euthanized with pentobarbital overdose, they underwent thoracotomy with their thoracic cavity exposed, pulmonary vasculature perfused via the pulmonary artery with cold D-hank's buffer, trachea surgically exposed and cannulated with a blunt 16-gauge needle, and lungs gently inflated with 10 mL cold D-Hank's buffer. The BAL was gently withdrawn from the lung and placed in a 50 mL centrifuge tube, which was repeated 3 times for each rat. The collected BAL was centrifuged (800 r/min) and the cells (precipitation) were resuspended in DMEM+10% FBS. The cells were counted with a hemocytometer and incubated for 2 h. The medium containing uncoated cells was poured out and the rest macrophages were continuously incubated.

Treatment of Rat Macrophages

Crystalline SiO_2 powder was heated at 180 °C for 1 h to prepare 100 μg/mL suspension which was added into the macrophage culture medium as previously described[12] and some macrophages were incubated in DMEM medium containing no SiO_2 suspension for 24 h. The culture media after SiO_2 treatment were collected and centrifuged (1500 r/min) to remove silica and 5 mL macrophage supernatant was then replaced with normal DMEM medium to culture lung fibroblasts for 24 h.

Identification of Transdifferentiation Proliferation of Rat Macrophages

MTT was dissolved in 5 mg/mL phosphate-buffered saline which was filtered and stored at 4 °C. The stock solution was diluted to a 0.5 mg/mL working solution. The medium was aspirated from cells and 200 μL MTT working solution was added into each well. The cells were incubated at 37 °C for 4 h, the MTT working solution was aspirated and the cells were subsequently lysed in 200 mL DMSO. The absorbance was read at 550 nm on the Biorad ELISA plate reader. Dosage range-finding experiments were performed at least 3 times for all

assays. Values in figures are expressed as percent of control groups.

RT- PCR

The cells were collected after treatment. RNA was isolated with Trizol reagent (Promega, USA) and reversed with cDNA synthesis kit (Transgen, Beijing, China). RNA integrity was analyzed by gel electrophoresis. RNA concentration in each sample was measured 3 times by spectrophotometry. The expressions of α-SMA, collagen I (COLI), collagen III (COLIII) mRNA, and GAPDH mRNA were detected by RT-PCR. The sequences of α-SMA, COLI, COLIII and GAPDH used for RT-PCR are 5'-CCGAGATCT CACCGACTACC-3' (sense) and 5'-TCCAGAGCGACATA GCACAG-3' (antisense), 5'-CCCACCCCAG CCGCAAAG AGT-3' (sense) and 5'-TTGGGTCCCTCGACTCCTACA-3' (antisense), 5'-TGCCCACAGCCTTCTACACCT-3' (sense) and 5'-CAGCCATTCCTCCCACTCCAG-3' (antisense), and 5'-GGTGCTGAGTATG TCGTGGAGT-3' (sense) and 5'-CAGTCTTCTGAGTGGCAGTGAT-3' (antisense), respectively. The length of PCR products was 121, 352, 240, and 292 bp, respectively. Forty-cycles of RT-PCR were performed. For each cycle, the sample was amplified at 94 °C for 4 min, denatured at 95 °C for 30 s, annealed at 61 °C for 45 s, extended at 72 °C for 45 s. and finally extended at 72 °C for 5 min.

Immunocytochemistry

Lung fibroblasts were cultured on glass culture slides stimulated with test substances for 24 h, washed in D-Hank's buffer, fixed in 20% acetone, and stored at 4 °C. α-smooth muscle actin (α-SMA) was detected with monoclonal mouse anti-rat smooth muscle actin polyclone at room temperature for 2 h in 1% BSA at the concentration of 1:500. Nonspecific staining was controlled by omitting primary antibodies and rat and mouse non-immune serum, respectively. Secondary antibody, goat anti-mouse IgG from Zhongshan (Beijing, China), was incubated at room temperature for 1 h in 1% BSA at the concentration of 1:1000. After washed with D-Hank's buffer, the slides were stained with DAB from Zhongshan (Beijing, China). Negative controls were included with secondary antibody alone.

ELISA

Data were analyzed using ELISA kits according to its manufacturer's instructions. Standard curves were plotted following the standards provided with the test kits. Calibration curves at 5 concentration points were plotted. CVs were 1%-5%. The OD value was measured with a spectrophotometer (Sunrise Remote/Touch Screen, TECAN, Austria).

Western Blot

Cell homogenates were prepared by sonicating the cells in 300 μL of ice-cold 50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 50 mmol/L NaF, 1 mmol/L Na_3VO_4, 5 mmol/L NaF EDTA, 50 mmol/L NaPPi, 1 μmol/L PMSF, 1 mmol/L DTT, 5 μg/mL Leupeptin, 2 μg/mL aprotinin and 1% NP-40, and centrifuged at 12 000 ×g for 30 min at 4 °C. The total protein concentration was measured by BCA assay. Samples (50 μg of total protein) were subjected to 10% SDS-PAGE and transferred to a nitrocellulose membrane (Hybond-ECL; Amersham Pharmacia Biotech, Buckinghamshire, UK). After blocked with non-fat dry milk (5% w/v), the membrane was incubated with antibodies specific for polyclonal rabbit anti-human c-raf (Cell Signaling Technology, Inc., Beverly, MA, USA). To confirm even loading, membranes were stripped and probed with β-actin antibody (Santa Cruz Biotechnology, Inc., Beverly, CA, USA). The protein bands were quantitated by densitometry and normalized using the β-actin signal. The protein bands were measured by Quantity One software for densitometric analysis.

Two-DE

Two-DE was performed as previously described[18]. In brief, proteins in each 450 μL, 150 μg aliquot of cell extracts were separated on a strip of immobilized pH gradient (pH range 3-10, 24 cm) by isoelectric focusing (IEF) in accordance with their isoelectric points (pI). The IEF was made at 30 V for 6 h, at 60 V for 6 h, at 150 V for 8 h, at 300 V for 1 h, at 600 V for 1 h, at 8000 V for 12 h, and at 50 V for 2 h. The strips were then equilibrated twice in equilibration buffer (6 mol/L urea, 2% SDS w/v, 50 mmol/L Tris-HCl buffer at pH 8.8 and 30% glycerol) containing 2% DTT or 2.5% iodoacetamide. The second dimension of electrophoresis was performed on a 12.5% polyacrylamide gel slab. The proteins were secondarily separated by SDS-PAGE at 3 w for 30 min and at 18 w for 10 h per gel in accordance with their molecular mass. After 2-DE, the gels were stained with silver nitrate as previously decribedl[19], and with coomassie blue for gel digestion.

Silver-stained 2D gels were scanned with an Amersham scanner of 300 dpi resolutions and analyzed with ImageMaster™ 2D Platinum software (Version 6.0; GE Healthcare). Protein spots were

detected automatically. Individual spot volume was normalized against total spot volume to obtain the volume percentages. After one or two small and sharp spots were selected manually as landmarks and one gel was chosen as the reference gel, alignment and auto-matching of the spots were carried out. Protein expression in different samples was analyzed by *t*-test. $P<0.05$ was considered statistically significant.

Gel Digestion of Interesting Protein

Different protein spots were harvested from Coomassie blue-stained gel. The gel samples were placed in a tube and washed twice with 500 μL and 250 μL ddH_2O for 15 min. For trypsin digestion, the gel samples were washed twice with 50 mmol/L NH_4HCO_3 and covered with 0.7 μL porcine trypsin solution (Promega, Madison, WI, USA) in 50 mmol/L NH_4HCO_3. After incubated overnight at 37 °C, the supernatant was removed into a second tube into which 40 μL 50 mmol/L w/v NH_4HCO_3 was added. Gel samples were washed with 40 μL of 50 mmol/L w/v NH_4HCO_3, and the supernatant was collected. The collected supernatants were mixed, the collected solution was washed with 70% v/v ACN and dried in a Speed Vac (Vacuum Concentrator, Bachhofer). The peptide mixture was desalted by ZipTip C-18 RP tips (Millipore, Billerica, MA, USA), wetted with 100% ACN and equilibrated with 0.1% TFA. Peptide samples redissolved in 10 mL 0.5% TFA were eluted with 50% ACN/0.1% TFA and dried in a Speed Vac (Vacuum Concentrator).

Identification of Peptide and MALDI-TOF-MS Mixture and Database Searching

The purified peptides were spotted on a MALDI plate and covered with 0.7 mL of 2 mg/mL 3, 5-dimethoxy-4-hydroxycinnamic acid matrix (Sigma) with 10 mmol/L $NH_4H_2PO_4$ in 60% ACN. All samples were analyzed by MALDI-TOF/TOF MS with a 4800 proteomics analyzer (Applied Biosystems, Foster City, CA). Monoisotopic peak masses were acquired in a mass range of 800-4000 Da, with a signal/noise ratio (S/N) of 200. Five of the most intense ion signals with common trypsin autolysis peaks and matrix ion signals excluded were selected as precursors for MS/MS acquisition. The peptide mass fingerprints (PMF) combined with MS/MS data were submitted to MASCOT version 2.1 (Matrix Science) for identification in database. The searching parameters were set as follows: trypsin cleavage (one missed cleavage allowed) as fixed modification, methionine oxidation as variable modification, peptide mass tolerance at 100 ppm, and fragment tolerance at 0.8 Da. The ion score confidence interval (CI %) for PMF and MS/MS data ≥95% was used as the criterion for successfully identified proteins .

Statistical Analysis

Data are presented as mean±SD. Significant difference between treatment and control groups was assessed by one-way analysis of variance (ANOVA) using SPSS 13.0. Statistical significance was determined by Fisher test. $P<0.05$ was considered statistically significant.

RESULTS

The OD value of lung fibroblasts was significantly higher after treatment than before dioxide silica treatment (0.42±0.14 *vs* 0.26±0.05, $P<0.05$).

The mRNA expression levels of α-SMA, COLI and COLIII were significantly higher in lung fibroblasts after treatment than before dioxide silica treatment ($P<0.05$, Table 1).

The protein expression levels of COLI and COLIII were significantly higher in lung fibroblasts after treatment than before dioxide silica treatment ($P<0.05$, Table 2).

The expression level of α-SMA was significantly higher in lung fibroblasts after treatment than before dioxide silica treatment ($P<0.05$, Figure 1), showing that lung fibroblasts are transformed to myofibroblasts.

The reproducible 2-DE images were analyzed by Image Master 2D Platinum 6.0 2-DE software (Figure 3). Over 246±31 protein spots were identified

Table 1. mRNA Level of α-SMA, COLI, and COLIII ($\overline{x} \pm s$)

Gene	No Stimulation	Stimulated	*P*
α-SMA	1	2.97±0.68	$P<0.05$
COLI	1	4.43±0.81	$P<0.05$
COLIII	1	3.30±0.46	$P<0.05$

Table 2. Protein Level of COLI and COLIII ($\overline{x} \pm s$)

Gene	No Stimulation	Stimulated	*P*
COLI (μg/L)	1.18±0.03	3.91±0.32$^{\Delta}$	$P<0.05$
COLIII (μg/L)	1.99±0.46	3.93±0.71$^{\Delta}$	$P<0.05$
Total Protein (μg/mL)	1.80±0.14	3.75±0.61$^{\Delta}$	$P<0.05$

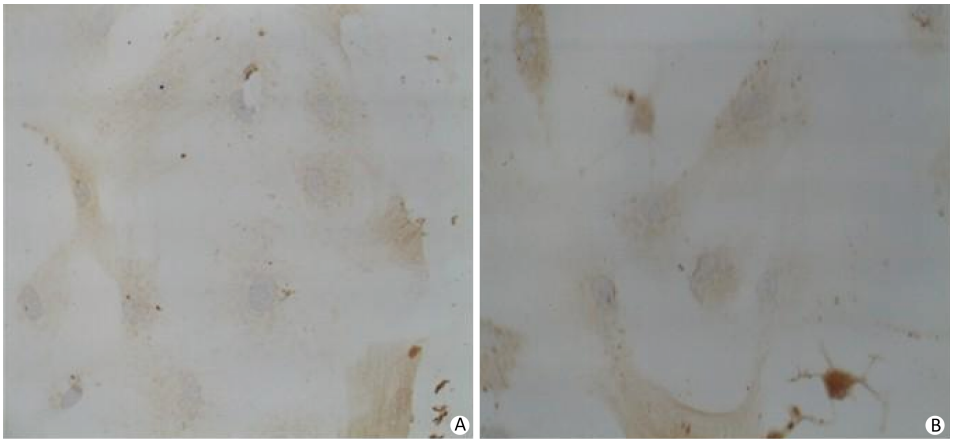

Figure 1. Immnunochemical patterns of α-SMA (×400) in control fibroblast (A) and α-SMA positive cells (B).

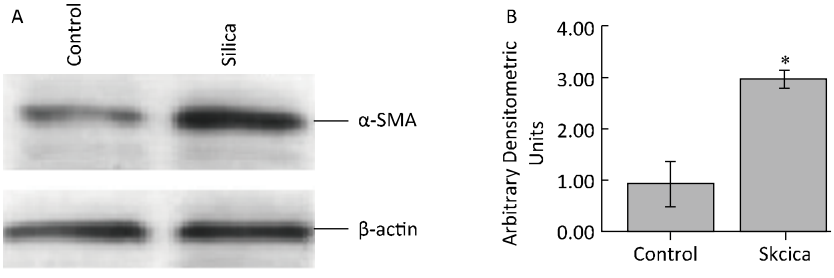

Figure 2. Effect of silica exposure on expression of α-SMA with β-actin as a loading control (A) and Protein level of α-SMA analyzed by Western blot (B).

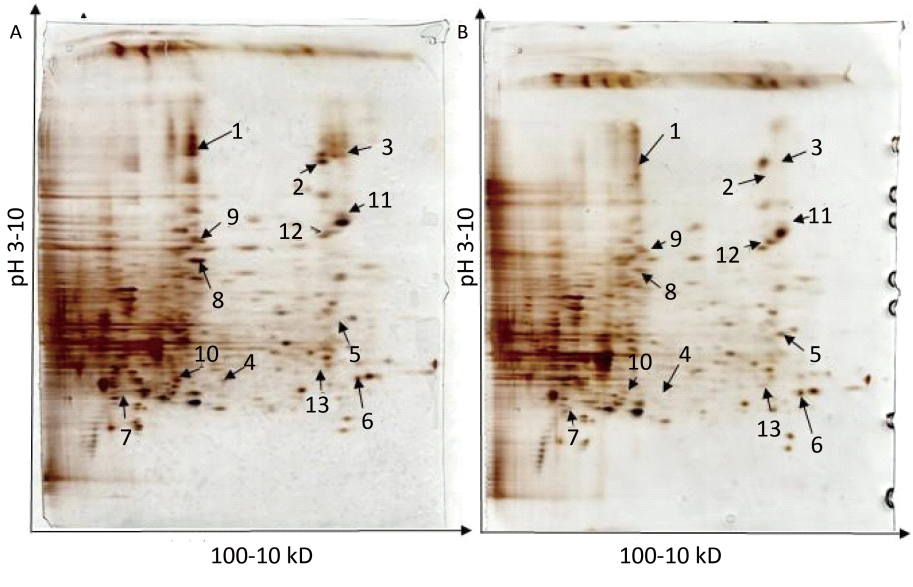

Figure 3. Protein expression 2-DE maps for normal rat lung fibroblasts (A) and dioxide silica- stimulated rat lung fibroblasts (B).

in a silver-stained 2-DE gel for the control group and 268±36 protein spots were identified for the silica group with an average matching rate of 80%. Thirteen proteins were differently expressed in silica group and control group. The expression level of 8 proteins (spots 1, 2, 3, 4, 8, 9, 10, and 11) was significantly higher than that of 2 proteins (spot 6 and 13) in silica group, only 3 proteins (spots 5, 7,

and 12) were expressed in control group.

Six proteins (spots 1, 2, 6, 9, 10, and 11) were incised randomly from gel, digested in gel with trypsin and obtained with MALDI-TOF-MS. The typical peptide masses were searched in NCBInr database by Mascot software, including vimentin, isoform CRA-b, glyceraldehyde 3-phosphate-ehydrogenase, peroxiredoxin 5, heterogeneous nuclear ribonucleoprotein A2, transgelin 2, and keratin K6 (Table 3, Figure 4).

DISCUSSION

Lung fibroblasts maintain a resting state under normal conditions. However, they are activated and transdifferentiated into myofibroblasts when exposed to crystalline silica, thus inducing α-SMA and collagen synthesis, affecting tissue repair and leading to occurrence and development of fibrosis[20]. During these processes, the cell proliferation ability and the protein and mRNA expression levels of α-SMA, COLI, and COLIII were significantly up-regulated (Figure 1B, Figure 2, indicating that the lung fibroblasts are transdifferentiated into activated myofibroblasts.

Transdifferentiation of fibroblasts in normal lungs into myofibroblasts characterized by expression of α-SMA is regarded as an early step in fibrotic process[21-22]. Myofibroblasts, a special kind of

Table 3. MALDI-TOF-MS-identified Different Expression Proteins in Lung Fibroblasts

Spot[a]	Protein Name[b]	Accession No.	Protein Score	Protein Score C.I.%	Ion Score	Total Ion C.I.%	Protein MW/pI	Peptide Count[c]
1	Heterogeneous nuclear Ribonucleoprotein A2	gi|157059859	719	100	492	100	36 088.9/8.67	23
2	Peroxiredoxin 5	gi|51261175	522	100	355	100	22 534.7/8.94	15
6	Keratin K6	gi|554464	78	99.886	60	99.98	3 105.6/5.55	1
9	Glyceraldehyde 3-phosphate-dehydrogenase	gi|56188	437	100	326	100	36 098.4/8.43	7
10	Vimentin, isoform CRA_b	gi|149021114	1020	100	713	100	53 725.1/5.06	30
11	Transgelin 2	gi|61557028	570	100	370	100	22 550.2/8.41	18

Note. a, the numbering and lettering corresponding to the two-dimensional gel electrophoresis image in Figure 1. b, accession number in NCBInr, SWISS-PROT. c, the number of matched peptide-covered protein sequences.

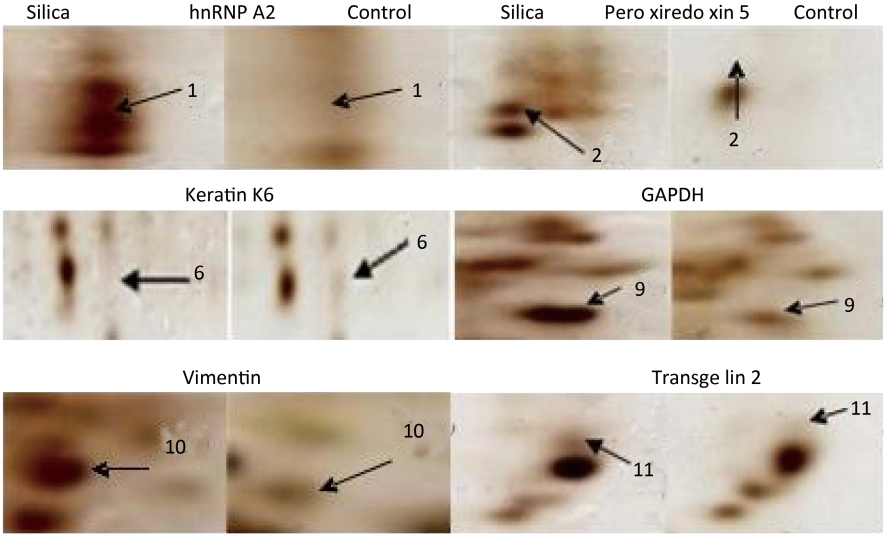

Figure 4. 2D-PAGE detection of andidate proteins in controls and silica activated fibroblasts. Arrows indicate the target proteins identified by MALDI-TOF and database searches. Left: silica-activated fibroblasts, right: controls.

fibroblasts in normal connective tissue, are widely distributed in normal tissues and organs, such as skin, eyes, lungs, heart, liver, kidney, and brain. The ultrastructure of myofibroblasts is of a special type between smooth muscle cells and fibroblasts[23]. Myofibroblasts can contract the actin-like structure, secrete collagens and extracellular matrix components. The main role of myofibroblasts in lung fibrosis is to produce extracellular matrix, promote wound healing, and secrete cytokines, growth factors and inflammatory mediators[5-6]. Detection of silica dust-induced protein changes in lung fibroblasts can help us understand more about the key proteins in fibroblasts and the mechanism underlying lung fibrosis.

In this study, 2-DE was used to identify disease-specific protein expression and compare gene expression profiles of state fibroblasts and silica-activated myofibroblasts on a proteome wide scale (Figure 3). The results demonstrated that different proteins can distinguish myofibroblasts from fibroblasts. Six proteins (spots 1, 2, 6, 9, 10, and 11) were selected to further identify the disease-specific protein expression, including vimentin, isoform CRA-b, glyceraldehyde 3-phosphate-dehydrogenase, eroxiredoxin 5, heterogeneous nuclear ribonucleoprotein A2, transgelin 2, keratin K6. It was reported that vimentin and transgelin 2 are a smooth muscle marker and induce smooth muscle differentiation[24-25]. It has been shown that most significantly increased genes are involved in development, extracellular matrix structure and turnover, and cellular growth and differentiation[16]. Moreover, vimentin, actin and smooth muscle are highly expressed in TGF-β_1-induced lung fibroblasts[26], which may explain the contractile properties of myofibroblasts. Vimentin, an intermediate filament, makes up the cytoskeleton along with microtubules and actin microfilaments. It was reported that the α-SMA and vimentin increase significantly when they are transited to myofibroblasts[27]. It has been shown that vimentin is expressed in stromal cells at week 1 and α-SMA is expressed in stromal cells at week 2 after photorefractive keratectomy[28]. Furthermore, α-SMA and vimentin are up-regulated as the biomarkers of myofibroblasts during epithelial-mesenchymal transition[27]. In a word, cytoskeleton proteins increase significantly when fibroblasts are activated.

Transgelin, a calponin-related protein, was found in smooth muscle and non-muscle cells in this study, showing that transgelin 2 is up-regulated when lung fibroblasts are transited to myofibroblasts. It was reported that transgelin can repress the expression of MMP 9 by reducing AP-1-dependent transactivation of the gene[29]. Transgelin, located in mouse alveolar epithelial type II cells, is a key regulator for alveolar epithelial type II cell migration with altered expression in experimental pulmonary fibrosis, which are consistent with our results. Therefore, transgelin can serve as an important regulator in lung fibrosis.

Heterogeneous nuclear ribonucleoprotein (hnRNP) is a RNA-binding protein, initially isolated from the nuclei of mammalian cells. More than 20 members of this protein family play an important role in mRNA synthesis, processing, and export[30]. In this study, the hnRNP A2 was up-regulated when the lung fibroblasts were activated. It was reported that hnRNP A2 is closely related with lung cancer[31-33] and juvenile idiopathic arthritis[34]. As a transcription coactivator, hnRNP A2 plays a role in the nuclear transcription response to mitochondrial respiratory stress[35]. The expression level of hnRNP H29 is significantly higher in adults than in fetuses[36]. The function of hnRNP A2 in silica-induced myofibroblasts is not known, but it may play an important role in fibroblast translation.

Peroxiredoxins exist ubiquitously in living organisms of bacteria, yeast, fungi and higher mammalian cells[37-39], whose function is to clear H_2O_2. In this study, peroxiredoxin 5 was up-regulated when the lung fibroblasts were transited to myofibroblasts. However, the exact role of peroxiredoxin is unclear in signal transduction, cellular proliferation, carcinogenesis and protein chaperoning among living cells. It was reported that peroxiredoxin 1 increases significantly in TGF-β_1-induced fibroblasts[40]. However, peroxiredoxin 2 oxidation is not related to the pathogenesis of IPF/UIP[41], which is contrary to our results. Therefore, the role of peroxiredoxin 5 in fibroblast activation should be further studied.

In conclusion, the approach presented in this study is considered to be suitable for studying the mechanism underlying lung fibroblast activation induced by silica. The differences in proteins between fibroblasts and myofibroblasts are complex and the functions of identified proteins are still unclear. Further study is needed to understand the mechanism of fibrosis.

REFERENCES

1. Nagalakshmi R, Nath J, Ong T, et al. Silica-induced micronuclei

and chromosomal aberrations in Chinese hamster lung (V79) and human lung (Hel 299) cells. Mutation research, 1995; 335(1), 27-33.
2. Fanizza C, Cinzia Lucia Ursinib, Emilia Pabaa, et al. Cytotoxicity and DNA-damage in human lung epithelial cells exposed to respirable alpha-quartz. Toxicology *in vitro*: an international journal published in association with BIBRA, 2007; 21(4), 586-94.
3. Kuempel ED, MD Attfield, V Vallyathan, et al. Pulmonary inflammation and crystalline silica in respirable coal mine dust: dose-response. Journal of biosciences, 2003; 28(1), 61-9.
4. Davis, GS. Pathogenesis of silicosis: current concepts and hypotheses. Lung, 1986; 164(3), 139-54.
5. Thannickal VJ, Toews GB, White ES, et al. Mechanisms of pulmonary fibrosis. Annual review of medicine, 2004; 55, 395-417.
6. Richards RJ, LC Masek, RF Brown. Biochemical and cellular mechanisms of pulmonary fibrosis. Toxicologic pathology, 1991; 19(4 Pt 1), 526-39.
7. Olbrück H, Seemayer NH, Voss B, et al. Supernatants from quartz dust treated human macrophages stimulate cell proliferation of different human lung cells as well as collagen-synthesis of human diploid lung fibroblasts *in vitro*. Toxicology letters, 1998; 96-97, 85-95.
8. Albrecht C, Borm PJ, Adolf B, et al. *In vitro* and *in vivo* activation of extracellular signal-regulated kinases by coal dusts and quartz silica. Toxicology and applied pharmacology, 2002; 184(1), 37-45.
9. Hetzel M, Bachem M, Anders D, et al. Different effects of growth factors on proliferation and matrix production of normal and fibrotic human lung fibroblasts. Lung, 2005; 183(4), 225-37.
10. Deng F, Guo X, Liu H, et al. Effects of dust storm PM2.5 on cell proliferation and cell cycle in human lung fibroblasts. Toxicology in vitro: an international journal published in association with BIBRA, 2007; 21(4), 632-8.
11. Gomperts BN, Strieter RM. Fibrocytes in lung disease. Journal of leukocyte biology, 2007; 82(3), 449-56.
12. Hinz B, Phan SH, Thannickal VJ, et al. The myofibroblast: one function, multiple origins. The American journal of pathology, 2007; 170(6), 1807-16.
13. Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest, 2007; 132(4), 1311-21.
14. Thiery JP, Acloque H, Huang RY, et al. Epithelial-mesenchymal transitions in development and disease. Cell, 2009; 139(5), 871-90.
15. Malmström J, Lindberg H, Lindberg C, et al. Transforming growth factor-beta 1 specifically induce proteins involved in the myofibroblast contractile apparatus. Molecular & cellular proteomics: MCP, 2004; 3(5), 466-77.
16. Hamilton RF Jr, Thakur SA, Holian A. Silica binding and toxicity in alveolar macrophages. Free radical biology & medicine, 2008; 44(7), 1246-58.
17. Kumar RK, Bennett RA, Brody AR. A homologue of platelet-derived growth factor produced by rat alveolar macrophages. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 1988; 2(7), 2272-7.
18. An J, Yuan Q, Wang C, et al. Differential display of proteins involved in the neural differentiation of mouse embryonic carcinoma P19 cells by comparative proteomic analysis. Proteomics, 2005; 5(6), 1656-68.
19. Yan JX, Wait R, Berkelman T, et al. A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry. Electrophoresis, 2000; 21(17), 3666-72.
20. Sappino AP, Schürch W, Gabbiani G. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Laboratory investigation: a journal of technical methods and pathology, 1990; 63(2), 144-61.
21. Phillips RJ, Burdick MD, Hong K, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. The Journal of clinical investigation, 2004; 114(3), 438-46.
22. De Wever O, Nguyen QD, Van Hoorde L, et al. Tenascin-C and SF/HGF produced by myofibroblasts *in vitro* provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 2004; 18(9), 1016-8.
23. Hwang IK, Park SM, Kim SY, et al. A proteomic approach to identify substrates of matrix metalloproteinase-14 in human plasma. Biochimica et biophysica acta, 2004; 1702(1), 79-87.
24. Larsen K, Malmström J, Wildt M, et al. Functional and phenotypical comparison of myofibroblasts derived from biopsies and bronchoalveolar lavage in mild asthma and scleroderma. Respiratory research, 2006; 7, 11.
25. Selman M, Pardo A, Barrera L, et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. American journal of respiratory and critical care medicine, 2006; 173(2), 188-98.
26. Willis BC, Liebler JM, Luby-Phelps K, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. The American journal of pathology, 2005; 166(5), 1321-32.
27. Chaurasia SS, Kaur H, de Medeiros FW, et al. Reprint of "Dynamics of the expression of intermediate filaments vimentin and desmin during myofibroblast differentiation after corneal injury". Experimental eye research, 2009; 89(4), 590-6.
28. Nair RR, Solway J, Boyd DD. Expression cloning identifies transgelin (SM22) as a novel repressor of 92-kDa type IV collagenase (MMP-9) expression. The Journal of biological chemistry, 2006; 281(36), 26424-36.
29. Yu H, Königshoff M, Jayachandran A, et al. Transgelin is a direct target of TGF-beta/Smad3-dependent epithelial cell migration in lung fibrosis. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 2008; 22(6), 1778-89.
30. Visa N, Alzhanova-Ericsson AT, Sun X, et al. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell, 1996; 84(2), 253-64.
31. Wurtz-T, Kiseleva E, Nacheva G, et al. Identification of two RNA-binding proteins in Balbiani ring premessenger ribonucleoprotein granules and presence of these proteins in specific subsets of heterogeneous nuclear ribonucleoprotein particles. Molecular and cellular biology, 1996; 16(4), 1425-35.
32. Katsimpoula S, Patrinou-Georgoula M, Makrilia N, et al. Overexpression of hnRNPA2/B1 in bronchoscopic specimens: a potential early detection marker in lung cancer. Anticancer research, 2009; 29(4), 1373-82.
33. Tomoum HY, Mostafa GA, El-Shahat EM. Autoantibody to heterogeneous nuclear ribonucleoprotein-A2 (RA33) in juvenile idiopathic arthritis: clinical significance. Pediatrics international: official journal of the Japan Pediatric Society,

2009; 51(2), 188-92.
34. Guha M, Pan H, Fang JK, et al. Heterogeneous nuclear ribonucleoprotein A2 is a common transcriptional coactivator in the nuclear transcription response to mitochondrial respiratory stress. Molecular biology of the cell, 2009; 20(18), 4107-19.
35. Navarro A, Rezaiekhaligh M, Keightley JA, et al. Higher TRIP-1 level explains diminished collagen contraction ability of fetal versus adult fibroblasts. American journal of physiology Lung cellular and molecular physiology, 2009; 296(6), L928-35.
36. Li S, Peterson NA, Kim MY, et al. Crystal Structure of AhpE from Mycobacterium tuberculosis, a 1-Cys peroxiredoxin. Journal of molecular biology, 2005; 346(4), 1035-46.
37. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature, 2000; 408(6809), 239-47.
38. Hofmann B, Hecht HJ, Flohé L. Peroxiredoxins. Biological chemistry, 2002; 383(3-4), 347-64.
39. Tian HY, Zhang KH, Gao X, et al. Comparative proteomic analysis of cell cycle-dependent apoptosis induced by transforming growth factor-beta. Biochimica et biophysica acta, 2009; 1794(10), 1387-97.
40. Shen ZJ, Esnault S, Rosenthal LA, et al. Pin1 regulates TGF-beta1 production by activated human and murine eosinophils and contributes to allergic lung fibrosis. The Journal of clinical investigation, 2008; 118(2), 479-90.
41. Vuorinen K, Ohlmeier S, Leppäranta O, et al. Peroxiredoxin II expression and its association with oxidative stress and cell proliferation in human idiopathic pulmonary fibrosis. The journal of histochemistry and cytochemistry: official journal of the Histochemistry Society, 2008; 56(10), 951-9.