Fibrotic gene expression coexists with alveolar proteinosis in early indium lung

Shuhei Noguchi, Masamitsu Eitoku, Hidenori Kiyosawa, and Narufumi Suganuma

Department of Environmental Medicine, Kochi Medical School, Kochi University, Kochi, Japan

Abstract

Occupational inhalation of indium compounds can cause the so-called “indium lung disease”. Most affected individuals show pulmonary alveolar proteinosis (PAP) and fibrotic interstitial lung disease. In animal experiments, inhalation of indium tin oxide or indium oxide has been shown to cause lung damage. However, the mechanisms by which indium compounds lead to indium lung disease remain unknown. In this study, we constructed a mouse model of indium lung disease and analyzed gene expression in response to indium exposure. Indium oxide (In₂O₃, 10 mg/kg, primary particle size <100 nm) was administered intratracheally to C57BL/6 mice (male, 8 weeks of age) twice a week for 8 weeks. Four weeks after the final instillation, histopathological analysis exhibited periodic acid-Schiff positive material in the alveoli, characteristic of PAP. Comprehensive gene expression analysis by RNA-Seq, however, revealed expression of fibrosis-related genes, such as surfactant associated protein D, surfactant associated protein A1, mucin 1, and collagen type I and III, was significantly increased, indicating that fibrotic gene expression progresses in early phase of indium lung. These data supported the latest hypothesis that PAP occurs as an acute phase response and is replaced by fibrosis after long-term latency.

Introduction

Indium compounds are materials used in the manufacture of transparent conductive films for flat panel displays (Yoshimura et al., 2013). Their demand has been rapidly increasing since the 2000s. Indium tin oxide (ITO) is the most commonly used compound; it consists of indium oxide and about 10% (wt) tin oxide.

Inhalation of indium compounds is known to cause respiratory damage. Occupational inhalation of ITO is linked to a new occupational disease named “indium lung disease” (Cummings et al., 2012). The first case of indium lung disease was confirmed in 2003, which manifested with interstitial lung disease accompanied by emphysema (Homma et al., 2003). This indium-induced emphysema resulted in the bilateral pneumothorax that eventually lead to the death of the patient, which raised the concern of the Industrial Safety and Health Department in Ministry of Health, Labor, and Welfare of Japan in 2004 (Ministry of Health, Labour and Welfare, 2004). Epidemiological studies of indium-exposed workers also revealed a dose-dependent relationship between indium exposure and lung disease characterized by interstitial fibrosis, emphysematous changes and alveolar inflammation (Chonan et al., 2007; Nakano et al., 2009, 2014). According to a report in 2012, at least 10 cases of indium lung disease had been reported worldwide (Cummings et al., 2012). Among these nine cases were exposed to ITO, whereas one case was exposed to indium oxide, which is a toxicological equivalent of ITO. Most of these cases were radiologically and pathologically diagnosed with interstitial fibrosis, however, some cases showed characteristic of fibrosis with pulmonary alveolar proteinosis (PAP). In two cases diagnosed as PAP in initial diagnosis, fibrosis developed during follow-up, indicating indium inhalation cause fibrosis after PAP. As we have one unpublished indium lung case caused by indium oxide exposure, we have focused the toxicity of indium oxide.

In animal experiments, intratracheal administration of ITO or indium oxide caused hamsters to develop respiratory disorders including fibrotic proliferation (Tanaka et al., 2002, 2010). Inhalation of either of ITO and indium oxide resulted in inflammation or PAP at the early stage, and long-term observation additionally showed fibrosis (Nagano et al., 2011a,b,c). Despite the histological data gathered in these animal experiments, these findings did not fully explain how indium lung disease begin with PAP and progress to fibrosis at the molecular level. In this study, we established an indium lung mouse model manifesting PAP by intratracheal administration of indium oxide to elucidate gene expression changes in the early phase of indium lung, or what we call early indium lung. We performed an exhaustive gene expression analysis by high-throughput sequencing, and RNA-sequencing (RNA-Seq) data were used to identify important genes in

Keywords

Fibrosis, indium oxide, indium lung disease, intratracheal administration, mouse model, pulmonary alveolar proteinosis, RNA-Seq
pulmonary proteinosis and fibrosis. Additionally, we explored other differentially expressed genes.

Materials and methods

Animals and indium oxide administration

To establish a mouse model of indium lung disease, 10 mg/kg of indium oxide (In2O3), primary particle size <100 nm, secondary particle size 462 ± 236 nm (Supplementary Figure S1) (Sigma, St. Louis, MO) was administered intratracheally to C57BL/6 mice (male, 8 weeks of age; Japan SLC, Inc., Shizuoka, Japan) twice a week for 8 weeks. For the control group, saline was intratracheally administered instead. Four weeks after the final administration, mice were euthanized and autopsied. All experiments were approved by the Ethical Committee for Animal Experimentation, Kochi Medical School, Kochi University, Japan.

Histopathology

The left lung was excised and immersed in 10% formalin, dehydrated, and embedded in paraffin. Four-micrometre tissue sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), or Masson trichrome.

RNA extraction and RNA-Seq

The right lung tissue was homogenized with TRI Reagent (Molecular Research Center Inc., Cincinnati, OH), and total RNA was extracted according to the manufacturer’s instructions. Library constructions and sequencing were performed as a custom service of Eurofins Genomics K.K. (Tokyo, Japan). Sequencing was performed using the Illumina HiSeq 2000 platform with a 2 × 100 bp module of version 3 chemistry. Sequencing results were mapped using the TopHat v2.0.13 software and the mouse genome reference sequence mm10. The mean mRNA expression value (FPKM) of each gene was calculated using the Cuffdiff program in the Cufflinks v.2.2.1 software. A q-value (false discovery rate–adjusted p value) <0.05 compared with the saline control sample was considered statistically significant, as calculated using Cuffdiff (Trapnell et al., 2012).

Results

Histopathology of the lungs of indium oxide-administered mice

The excised lung tissue from indium oxide administered mice was stained with H&E or PAS (Figure 1) and observed microscopically. Presumed indium oxide particles were evident in some areas. There were also areas of cellular infiltration were evident. The accumulation of PAS-positive material was evident in the alveoli, characteristic of PAP. However, no cholesterol clefts or interstitial fibrosis were observed.

RNA-Seq analysis in indium oxide-administered mice

RNA-Seq analysis was performed on the homogenized lung tissue of indium oxide-administered mice to examine changes in gene expression compared with saline control mice (n = 4 per group) (Figure 2) (DDBJ Sequence Read Archive accession number DRA DRA004749). The average number of total reads was 40,408,945, and on average 88.7% of each fragment was mapped. In total, 3730 genes showed significant differences in expression between the indium oxide and control samples. These genes included the PAP- or fibrosis-related genes (Supplementary Table S1).

PAP-related gene expression

The expression of Csfr2, also known as granulocyte-macrophage colony-stimulating factor (GM-CSF), was significantly increased in indium oxide-administered mice. The expression of Csfr2ra, Csfr2rb, and Csfr2rb2, receptors of Csfr2, were also significantly increased in indium oxide-exposed mice (Figure 3a–d). The expression of the genes downstream of Csfr2, namely Spi1 (PU.1), Cd14, Cd180 (RP105), and Irap3 (interleukin-1 receptor-associated kinase 3), were also increased in expression (Figure 3e–h).

Fibrosis-related gene expression

The expression of the Muc1 (mucin 1), Sftpd (surfactant protein D), and Sftpal1 (surfactant protein A1) genes, known fibrosis marker protein coding genes, was significantly increased in indium oxide-administered mice (Figure 4a and c). The expression of Tgfb1 (transforming growth factor beta 1), a key factor in fibrosis, was also increased. Expression of the Smad genes, Smad6 and Smad7, was decreased (Figure 4b). Expression of the collagen genes, Col1a1 (collagen type I A1), Col1a2 (collagen type I A2), and Col3a1 (collagen type III A1), that accumulate in fibrosis was significantly increased in indium oxide-administered mice (Figure 4a). Timp1 (tissue inhibitor of metalloproteinase 1), another fibrosis-related gene was also increased in expression in exposed mice (Figure 4d). Tjp1 (tight junction protein 1), an epithelial marker known to be decreased in fibrosis, was significantly decreased in expression (Figure 4e), by contrast, the expression of mesenchymal genes such as S100a4 (S100 calcium binding protein A4), and Vim (vimentin) was increased in indium oxide-administered mice (Figure 4f and g). Some genes showed no significant difference in expression between control and indium oxide-administered mice including the mesenchymal and epithelial marker genes Acta2 (α-SMA), and Cdh1 (E-cadherin), respectively (Figure 4h and i). Expression of the mesenchymal marker Cdh2 (N-cadherin), however, was significantly decreased (Figure 4j).

Other genes significantly changed in expression

The expression of inflammatory cytokine-related genes, such as Il1b (interleukin-β1), Il6 (interleukin 6), and Tnf (tumour necrosis factor), was increased in indium oxide-administered mice (Figure 5a). The gene expression levels of chemokines, such as Ccl4 (chemokine C-C motif ligand 4), and Cxcl10 (chemokine C-X-C motif ligand 10), were also significantly increased in indium oxide-administered mice (Figure 5a). The expression of oxidative stress marker genes, Ptgs2 (known as COX2), Sod2 (superoxide dismutase 2, mitochondrial), and Gpx1 (glutathione peroxidase 1), was significantly increased (Figure 5b). Ear1 and Ear2,
eosinophil-associated, ribonuclease A family genes, were highly expressed in saline control mice but showed decreased expressions in indium oxide-administered mice (Supplementary Table S1). The expression of some non-coding RNAs was also affected, for example, Fendrr (Foxf1 adjacent non-coding developmental regulatory RNA) expression was significantly decreased in indium oxide-administered mice (Figure 5c).

**Discussion**

This is the first study to report comprehensive gene expression analysis using RNA-Seq in a mouse model of indium lung disease, which we established using intratracheal instillation. Animal models have previously been employed to investigate indium lung disease using either intratracheal or inhalational administrations. Although the toxicity of indium...
oxide was first reported in 1961 (Leach et al., 1961), that of ITO was reported in 2002 after first patient of indium lung disease died. In this report, 8-week-old hamsters were administered 6.0 mg/kg of ITO particles by intratracheal instillation along with ether once a week, for a total of 16 times. Exposure to ITO resulted in an inflammatory response, with the infiltration of alveolar macrophages, necrotic cell debris, and inflammatory cells into the lung and the accumulation of alveolar macrophages in the alveolar spaces (Tanaka et al., 2002). Another report using the same animal model investigated indium lung disease using indium oxide instead of ITO. They administered 2.7 mg/kg or 5.4 mg/kg of indium oxide to hamsters intratracheally twice a week for 8 weeks. Using the higher dose, they observed interstitial fibrotic proliferation 40 weeks after the final instillation (Tanaka et al., 2010). This type of cellular proliferation was not however evident 16 weeks after the final instillation, suggesting that some effects of indium oxide exposure may have long latency. In a separate study using a mouse model, mice were exposed to ITO or indium oxide aerosol for 6 hours/day, 5 days/week for 2 weeks at a concentration of 10 or 100 mg/m$^3$ using inhalation exposure chambers, and PAP was observed in the lungs after the exposure duration (Nagano et al., 2011b). In the current study, we focused indium oxide known as one of the causes of indium lung disease, and administered indium oxide into mice

![Figure 2. Gene expression analysis of the lung tissue from indium oxide-administered mice compared with control mice. Mean mRNA expression value (FPKM) in each group of mice administered either saline or In$_2$O$_3$ was measured by HiSeq. FPKM was calculated using the Cuffdiff program. Circle: genes not significantly changed in expression, cross: genes significantly changed in expression ($q<0.05$). The x- and y-axes indicate common logarithm of FPKM. FPKM values lower than 0.01 are shown as −2 with logarithmic processing.]

![Figure 3. Gene expression of PAP-related genes in early indium lung. Results of RNA-Seq for PAP-related genes. (a) Csf2, (b) Csf2ra, (c) Csf2rb, (d) Csf2rb2, (e) Spi1, (f) Cd14, (g) Cd180, and (h) Irak3 gene expression values were represented by FPKM. *$q<0.05$ compared with saline control sample, calculated using Cuffdiff.]

S. Noguchi et al. Inhal Toxicol, 2016; 28(9): 421–428
Figure 4. Gene expression of fibrosis-related genes in early indium lung. Results of RNA-Seq for fibrosis-related genes. Heat map of significantly changed gene expression in each gene category. (a) Collagen trimer (GO:0005581), (b) transforming growth factor beta receptor signaling pathway (GO:0007179), (c) Muc1, (d) Timp1, (e) Tgf1, (f) S100a4, (g) Vim, (h) Acta2, (i) Cdh1, and (j) Cdh2 gene expression values were represented by FPKM. *q<0.05 compared with saline control sample, calculated using Cuffdiff.

Figure 5. Gene expression of inflammatory or oxidative stress-related genes and non-coding RNAs in early indium lung. Heat map of significantly changed gene expression in each gene category (q<0.05). (a) Inflammatory response (GO:0006954), (b) response to oxidative stress (GO:0006979), and (c) non-coding RNA-related gene expression.
intratracheally in the same schedule with previous study which used hamsters (Tanaka et al., 2010). The concentration of indium oxide was 10 mg/kg, about two times higher than this previous study. Our preliminary study revealed the significant increasing of relative lung weight in 10 mg/kg comparing other lower concentrations (0, 1.25, 2.5, and 5 mg/kg indium oxide), which suggests that inflammation is clearly caused in this concentration. This is the first report of mouse model of indium lung disease using intratracheal administration method, although there is the limitation that the character of particle, dose and mode of exposure would be different with the situation in the workplace handling indium. In our present study, histopathology of the lung tissue revealed PAP and the accumulation of PAS-positive material in the alveoli, confirming the findings of previous studies on indium lung disease. These findings confirmed that intratracheal administration and inhalation via exposure chambers both provided similar results suggesting that intratracheal administration is a cost-effective method to study indium lung disease in the mouse model. Furthermore, mice have been better defined genetically than other rodent species, i.e. hamsters and rats. Therefore, we proposed that this mouse model was the most suitable for analyzing the genetic mechanisms involved in indium lung disease.

PAP was first described in 1958 (Rosen et al., 1958), and by 2002, over 400 cases were reported (Seymour & Presneill, 2002). PAP occurs in three major distinct forms. The first form is congenital PAP caused by mutation of surfactant protein genes (Seymour & Presneill, 2002). The second one is autoimmune PAP caused by anti-GM-CSF auto-antibodies (Borie et al., 2011; Carey & Trapnell, 2010; Huffman et al., 1996; Kitamura et al., 1999). The last one is PAP that does not fit in the other two categories, for example, lysinuric protein intolerance, immunodeficiency disorders (Seymour & Presneill, 2002), and caused by inhalation of materials, such as silica (Xipell et al., 1977), aluminium (Miller et al., 1984), titanium (Keller et al., 1995). Mechanisms of congenital and autoimmune PAP (Trapnell et al., 2003) are well known, however, there are little information about molecular mechanism of PAP caused by inhalation of inorganic materials (Badding et al., 2014, 2015; Lison et al., 2009). In order to confirm indium-induced PAP in our mouse model is not autoimmune PAP, we analyzed the expression of genes related to GM-CSF pathway. GM-CSF is a glycoprotein cytokine that stimulates proliferation and maturation of macrophages (Gasson, 1991) and is necessary for surfactant clearance (Yoshida et al., 2001). Anti-GM-CSF antibodies neutralize GM-CSF and inhibit the downstream of the GM-CSF pathway, resulting in the impairment of the surfactant clearance by alveolar macrophage (Trapnell et al., 2003). Csf2 (encoding GM-CSF) gene expression was found to be increased in the lungs of mice exposed to indium oxide. Additionally, the expression of genes downstream of the GM-CSF pathway, the receptors of Csf2, and downstream genes of GM-CSF pathway, namely Spi1 (encoding Pu.1), Cdi4, Cdi180, and Irak3 (Carey & Trapnell, 2010; Trapnell & Whitsett, 2002), were also increased. These results suggested that the GM-CSF pathway is intact and correspond with the findings in many human indium lung disease cases. Acute silicosis, fibrosis caused by silica exposure, is recognized as silicoproteinosis because of histologic resemblance to PAP (Castranova & Vallyathan, 2000; Xipell et al., 1977). Silicoproteinosis is seen as a form of not congenital or autoimmune PAP (Xiao et al., 2015). Early indium lung in our model also shows histopathological features of PAP, which would not be congenital or autoimmune PAP. This study will be a starting point to understand the molecular mechanism of exposure-related PAP.

In pulmonary fibrosis, TGF-β1 is an important key regulator in the progression of fibrosis. In our mouse model of indium lung disease, Tgfb1 (encoding TGF-β1) gene expression was significantly increased. By contrast, Smad6 and Smad7, downstream inhibitors of the TGF-β1 signaling pathway (Schmierer & Hill, 2007), were significantly decreased in expression. These Smad genes have previously been reported to be decreased in expression in a bleomycin-induced fibrosis mouse model (Peng et al., 2013). Additionally, the expression of type I and type III collagen genes, known to accumulate during fibrosis, was significantly increased in the indium lung disease model. However, no collagen deposits were detected in indium oxide-administered mice by histopathology. Genes encoding proteins used as a biomarkers of fibrosis, Timp1 (Peng et al., 2013), Sl10a4 (Tanjore et al., 2009), and Vim (Kalluri & Weinberg, 2009) were also significantly increased in expression, as were Muc1, Sftp1, and Sftp1, the blood markers of fibrosis Krebs von den Lungen (KL-6), sialylated carbohydrate antigen related to Muc1, surfactant protein (SP)-A, and SP-D cording genes, respectively (Greene et al., 2002; Ishikawa et al., 2012; Kuroki et al., 1998; Samukawa et al., 2012). High level of serum KL-6 was observed in the patient of indium lung disease (Homma et al., 2005) and indium-processing workers (Chonan et al., 2007). These results suggested that the process of fibrosis had been initiated in terms of the gene expression profile but not on the histopathological features in early indium lung. The expression of several epithelial or mesenchymal marker genes, such as Cdh1 or Acta2, was not changed suggesting that epithelial-to-mesenchymal transition, a key phenomenon in fibrogenesis (Kalluri & Weinberg, 2009; Noguchi et al., 2015), had not occurred or was undetectable, possibly due to RNA extraction from the whole lung. In histopathology, there were no collagen accumulation characteristics of pulmonary fibrosis, and there were no cholesterol clefts, observed in many human indium lung cases (Cummings et al., 2012), and observed in long-term observation of ITO- or indium oxide-treated hamsters (Tanaka et al., 2010). That is, fibrosis did not develop completely but partially at the gene expression level. Although our data on PAP and fibrosis-related gene expression indicated partial PAP and fibrosis in the subacute phase, they also supported the latest hypothesis that fibrosis occurred after PAP in indium-exposed human cases (Cummings et al., 2012). This is the first report to reveal that exposure of indium compounds causes fibrotic gene expression changes as well as the histopathology of PAP in early indium lung. Long-term observations in our indium lung model may uncover the histopathological features of fibrosis.

Gene expression changes were observed not only in genes directly related to fibrosis or proteinosis. In our model,
inflammatory cytokines were also significantly increased in indium oxide-administered mice. The expression of chemokine genes, such as Ccl4 and Cxcl10 (Solomon et al., 2013), was also increased. These findings indicated that indium oxide exposure can cause inflammation as shown in previous in vitro studies (Badding et al., 2015; Jeong et al., 2015). 

*Chil3*, a gene known to be abundant in the mouse lung and increased during allergy (Hung et al., 2002; Webb et al., 2001), was significantly decreased in expression in indium oxide-administered mice. Although the fibrosis- or PAP-related function of this gene is unknown, it may be related to mechanism of the indium lung. Expression of *Ear1* and *Ear2* was also significantly decreased in indium oxide-administered mice. While these two genes have previously been shown to be increased in expression during pulmonary inflammation (Cormier et al., 2002), they may not be required in the inflammatory response caused by indium exposure. The expression of oxidative stress-related genes was significantly increased in our model suggesting that indium oxide exposure induces oxidative stress, a known cause of pulmonary fibrosis (Kinnula et al., 2005; Lison et al., 2009; Liu et al., 2010, 2012). It is clear that inflammation and the oxidative stress response contributed to the development of fibrosis, suggesting that indium oxide administration promoted fibrosis. Moreover, there were many non-coding RNAs that displayed significantly changed expression. For example, non-coding RNA *Fendrr* showed a significant decrease in expression; this non-coding RNA is related to heart development (Grote et al., 2013) and pulmonary fibrosis (Sakamoto et al., 2014). These results indicated the possibility that many more non-coding RNAs including imprinted genes, such as *Peg13*, *Meg3*, and *Kcnq1ot1*, are involved in PAP or fibrosis. RNA-Seq analysis revealed many genes that were changed in expression that were not known as PAP- or fibrosis-related genes. Therefore, this method may facilitate the identification of new genes related to indium lung disease.

**Conclusions**

In summary, we have established a mouse model of indium lung disease using intratracheal administration of indium oxide. Histopathology of mouse lung tissue excised in the subacute phase revealed that PAS-positive material accumulated in the alveoli space, confirming PAP. From gene expression analysis, the data for PAP-related genes indicated that the GM-CSF pathway is intact in indium oxide-exposed mice. Fibrosis-related genes were differentially expressed in indium oxide-exposed mice compared with control mice. These data suggested that fibrosis had been initiated in early indium lung under the subacute PAP response, as predicted by human indium lung disease cases. Early detection of PAP signal will enable early treatment of disease by inhalation of indium.

**Acknowledgements**

The authors thank Dr. Ichiro Murakami for expert advice on histopathological analysis.

**Declaration of interest**

The authors declare that they have no conflicts of interest.

This work was supported by JSPS KAKENHI Grant Number 24790588.

**References**

Badding MA, Fix NR, Antonini JM, Leonard SS. (2014). A comparison of cytotoxicity and oxidative stress from welding fumes generated with a new nickel-, copper-based consumable versus mild and stainless steel-based welding in RAW 264.7 mouse macrophages. PLoS One 9:e101310.

Badding MA, Schwegler-Berry D, Park J-H, et al. (2015). Sintered indium-tin oxide particles induce pro-inflammatory responses in vitro, in part through inflammasome activation. PLoS One 10:e0124368.

Borie R, Danel C, Debray M-P, et al. (2011). Pulmonary alveolar proteinosis. Eur Respir Rev 20:98–107.

Carey B, Trapnell BC. (2010). The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 135:223–35.

Castranova V, Vallyathan V. (2000). Silicosis and coal workers' pneumoconiosis. Environ Health Perspect 108:675–84.

Chonan T, Taguchi O, Omae K. (2007). Interstitial pulmonary disorders in indium-processing workers. Eur Respir J 29:317–24.

Cormier SA, Yuan S, Crosby JR, et al. (2002). TH2-mediated pulmonary inflammation leads to the differential expression of ribonuclease genes by alveolar macrophages. Am J Respir Cell Mol Biol 27: 678–87.

Cummings KJ, Nakano M, Omae K, et al. (2012). Indium lung disease. Chest 141:1512–21.

Gasson JC. (1991). Molecular physiology of granulocyte-macrophage colony-stimulating factor. Blood 77:1131–45.

Greene KE, King Jr TE, Kuroki Y, Bucher-Bartelson B, et al. (2002). Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. Eur Respir J 19:439–46.

Grote P, Wittler L, Hendrix D, et al. (2013). The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 24:206–14.

Homma S, Miyaamoto a, Sakamoto S, et al. (2005). Pulmonary fibrosis in an individual occupationally exposed to inhaled indium-tin oxide. Eur Respir J 25:200–4.

Homma T, Ueno T, Sekizawa K, et al. (2003). Interstitial pneumonia developed in a worker dealing with particles containing indium-tin oxide. J Occup Health 45:137–9.

Huffman JA, Hull WM, Dranoff G, et al. (1996). Pulmonary epithelial cell expression of GM-CSF corrects the alveolar proteinosis in GM-CSF-deficient mice. J Clin Invest 97:649–55.

Hung S-I, Chang AC, Kato I, Chang N-CA. (2002). Transient expression of Ym1, a heparin-binding lectin, during developmental hematopoi- esis and inflammation. J Leukoc Biol 72:72–82.

Ishikawa N, Hattori N, Yokoyama A, Kohno N. (2012). Utility of KL-6/ MUC1 in the clinical management of interstitial lung diseases. Respir Investig 50:3–13.

Jeong J, Kim J, Seok SH, Cho W-S. (2015). Indium oxide (In2O3), a gene known to be abundant in the mouse lung and 

Kalluri R, Weinberg RA. (2009). The basics of epithelial-mesenchymal transition. J Clin Investig 119:1420–8.

Keller CA, Frost A, Cagle PT, Abraham JL. (1995). Pulmonary alveolar proteinosis in a painter with elevated pulmonary concentrations of titanium. Chest 108:277–80.

Kinnula VL, Fattman CL, Tan RJ, Oury TD. (2005). Oxidative stress in pulmonary fibrosis: a possible role for redox modulatory therapy. Am J Respir Crit Care Med 172:417–22.

Kitamura T, Tanaka N, Watanebe J, et al. (1999). Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med 190:875–80.

Kuroki Y, Takahashi H, Chiba H, Akino T. (1998). Surfactant proteins A and D: disease markers. Biochim Biophys Acta 1408:334–45.

Leach LJ, Scott JK, Armstrong RD, et al. (1961). The inhalation toxicity of indium sesquioxide in the rat. ORINS Rep US Energy Comm UR- 590:1–30.
Lison D, Laloy J, Corazzari I, et al. (2009). Sintered indium-tin-oxide (ITO) particles: a new pneumotoxic entity. Toxicol Sci 108: 472–81.

Liu HH, Chen CY, Chen GI, et al. (2012). Relationship between indium exposure and oxidative damage in workers in indium tin oxide production plants. Int Arch Occup Environ Health 85:447–53.

Liu H-H, Lin M-H, Chan C-I, Chen H-L. (2010). Oxidative damage in foundry workers occupationally co-exposed to PAHs and metals. Int J Hyg Environ Health 213:93–8.

Miller RR, Churg AM, Hutcheon M, Lam S. (1984). Pulmonary alveolar proteinosis and aluminum dust exposure. Am Rev Respir Dis 130: 312–15.

Ministry of Health, Labour and Welfare. (2004). Technical guideline for preventing health impairment of workers engaged in the indium tin oxide handling processes. Government of Japan. Available from: http://www.mhlw.go.jp/bunya/roudoukijun/anzeneisei42/dl/03.pdf. [Last accessed: 6 Feb 2016].

Nagano K, Gotoh K, Kasai T, et al. (2011a). Two- and 13-week inhalation toxicities of indium-tin oxide and indium oxide in rats. J Occup Health 53:51–63.

Nagano K, Nishizawa T, Eitaki Y, et al. (2011b). Pulmonary toxicity in mice by 2- and 13-week inhalation exposures to indium-tin oxide and indium oxide aerosols. J Occup Health 53:234–9.

Nagano K, Nishizawa T, Umeda Y, et al. (2011c). Inhalation carcinogenicity and chronic toxicity of indium-tin oxide in rats and mice. J Occup Health 53:175–87.

Nakano M, Omae K, Tanaka A, et al. (2009). Causal relationship between indium compound inhalation and effects on the lungs. J Occup Health 51:513–21.

Nakano M, Omae K, Uchida K, et al. (2014). Five-year cohort study: emphysematous progression of indium-exposed workers. Chest 146: 1166–75.

Noguchi S, Eitoku M, Moriya S, et al. (2015). Regulation of gene expression by sodium valproate in epithelial-to-mesenchymal transition. Lung 193:691–700.

Peng R, Sridhar S, Tyagi G, et al. (2013). Bleomycin induces molecular changes directly relevant to idiopathic pulmonary fibrosis: a model for ‘active’ disease. PLoS One 8:e59348.

Rosen SH, Castleman B, Liebow AA, et al. (1958). Pulmonary alveolar proteinosis. N Engl J Med 258:1123–42.

Sakamoto K, Yu G, Maya JD, et al. (2014). Expression of Fendrr, a developmentally regulated long noncoding RNA, is attenuated in pulmonary fibrosis. Am J Respir Crit Care Med 189:A3653.

Samukawa T, Hamada T, Uto H, et al. (2012). The elevation of serum napsin A in idiopathic pulmonary fibrosis, compared with KL-6, surfactant protein-A and surfactant protein-D. BMC Pulm Med 12:55.

Schmierer B, Hill CS. (2007). TGF-beta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol 8:970–82.

Seymour JF, Presneill JJ. (2002). Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med 166:215–35.

Solomon GM, Frederick C, Zhang S, et al. (2013). IP-10 is a potential biomarker of cystic fibrosis acute pulmonary exacerbations. PLoS One 8:e72398.

Tanaka A, Hirata M, Homma T, Kiyohara Y. (2010). Chronic pulmonary toxicity study of indium-tin oxide and indium oxide following intratracheal instillations into the lungs of hamsters. J Occup Health 52:14–22.

Tanaka A, Hirata M, Omura M, et al. (2002). Pulmonary toxicity of indium-tin oxide and indium phosphate after intratracheal instillations into the lung of hamsters. J Occup Health 44: 99–102.

Tanjore H, Xu XC, Polosukhin VV, et al. (2009). Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. Am J Respir Crit Care Med 180:657–65.

Trappell BC, Whitsett JA. (2002). GM-CSF regulates pulmonary surfactant homeostasis and alveolar macrophage-mediated innate host defense. Annu Rev Physiol 64:775–802.

Trappell BC, Whitsett JA, Nakata K. (2003). Pulmonary alveolar proteinosis. N Engl J Med 349:2527–39.

Trappell C, Roberts A, Goff L, et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7:562–78.

Webb DC, McKenzie ANJ, Foster PS. (2001). Expression of the Ym2 lectin-binding protein is dependent on interleukin (IL)-4 and IL-13 signal transduction: identification of a novel allergy-associated protein. J Biol Chem 276:41969–76.

Xiao Y-L, Xu K-F, Li Y, et al. (2015). Occupational inhalational exposure and serum GM-CSF autoantibody in pulmonary alveolar proteinosis. Occup Environ Med 72:504–12.

Xipell JM, Ham KN, Price CG, Thomas DP. (1977). Acute silicoproteinosis. Thorax 32:104–11.

Yoshida M, Ikegami M, Reed JA, et al. (2001). GM-CSF regulates protein and lipid catabolism by alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 280:L379–86.

Yoshimura A, Daigo I, Matsuno Y. (2013). Global substance flow analysis of indium. Mater Trans 54:102–9.