



Persistent Expression Changes of Fibrosis Related Genes in the Lung Tissues of Rats Exposed to Lunar Dust Particles

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INTRODUCTION

The Moon's surface is covered by a layer of reactive dust, containing 1-2% of respirable fine dust (< 3 μm). The habitable area of any lunar landing vehicle would inevitably be contaminated with lunar dust that could pose a health risk. The purpose of the study is to evaluate the toxicity of Apollo moon dust in rodents through inhalation to assess the health risk of dust exposures to humans and to identify the mechanisms and potential pathways involved in lunar dust-induced toxicity.

MATERIAL AND METHODS

1. **Animals and dust exposure:** Pathogen-free Fischer 344 adult male rats (150-250 g; ~8-10 weeks old at arrival) were purchased from Charles River and were allowed to acclimate for 1 week before they were used in experiments. In each experiment, 132 male Fischer rats were randomly divided into different dose groups (control air, 2.1, 6.8, 21, or 61 mg/m³) and were placed in Battelle rat restraint tubes, which were then connected to the 24 nose-ports of a Jaeger-NYU nose-only chamber. All animals were exposed 6 hours daily, 5 days a week for 4 weeks for a total of 120 hours. After each daily 6-h exposure, the animals were returned to the vivarium, housed in pairs, and observed for clinical signs.

2. **Collection and assessment of bronchoalveolar lavage (BAL) cells:** At day 1, day 7, 4 week, and 13 week post the last exposure, five animal per dose group were sacrificed. The bronchoalveolar lavage fluid (BALF) was collected by lavaging the right lung lobes with a total of 24 ml of phosphate-buffered saline (PBS) in five times. The lavage was centrifuged, and the cell pellets were suspended in 1 ml of HEPES-buffered solution. Zymosan induced luminol based chemiluminescence assay was used to assess the activity of BAL cells.

3. **Tissue collection and gene expression analysis:** The lavaged lung tissue was snap frozen in LN2. The expressions of 84 fibrosis related genes were analyzed using the RT2 Profiler PCR Array technique. After DNase treatment, 1 μg total RNA of each of the three samples per time-point and per group animals that were exposed to control air or high dose lunar dust particles (60 mg/m³) was used to synthesize cDNA using a RT2 PCR array first strand kit. Real-time PCR Analysis on 18 Genes of Interest (Table 1)

RESULTS

1. Expression changes in fibrosis related genes after exposure to 61mg/m³ lunar dust particles:

Figure 1 shows the fold changes of gene expressions in the lung tissue of the animals exposed to 61mg/m³ lunar dust in comparison to the controls at 13 weeks post-exposure.

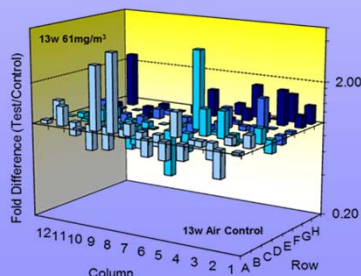


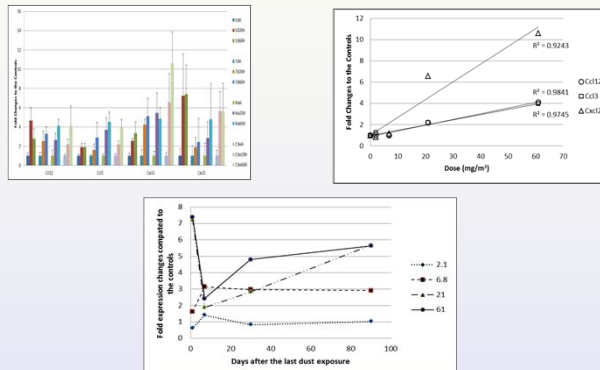
Table 1. List of genes that were significantly up- or down-regulated in the lung tissue after lunar dust exposure. (p<0.05, n=3 per control or 61mg/m³ dust exposed group; †/‡: fold change between 1.5 to 2.0; ††/‡‡: fold change > 2). Genes of interest were highlighted.

	24hr	7 days	4 weeks	13 weeks
Agt	-	-	-	↓
Bmp7	-	↓	↓	-
Cav1	-	↓	↓	-
Ccl12	↑↑	↑↑	↑↑	↑↑
Ccl3	↑↑	↑↑	↑↑	↑↑
Dcn	-	-	↓	-
Edn1	-	↑	-	↑
Eng	-	↓	↓	-
Hgf	-	-	↓	↓
Il1a	-	-	↓	↑
Il1b	-	↑	-	↑
Itga1	-	-	↓	-
Itgb5	-	-	↓	-
Itgb8	↑↑	↑↑	↑↑	↑↑
Mmp13	-	↓	↓	↓
Mmp8	-	↑	-	-

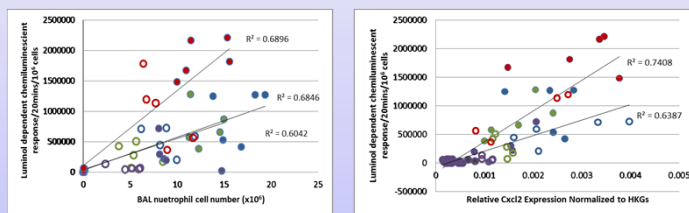
	24hr	7 days	4 weeks	13 weeks
Myc	-	-	↑	-
Pdgfra	-	-	↓	-
Pdgfrb	-	-	↓	-
Plau	-	-	↓	↓
Plg	-	-	↓	-
Serpine1	-	-	↑	↑↑
Smad6	-	-	↓	↓
Snai1	-	-	↓	↓
Tgfb1	-	-	↑	↑
Thbs1	-	-	↑	↑
Thbs2	-	-	↑↑	-
Tnf	↑↑	↑↑	↑↑	↑↑
Vegfa	-	-	↑	↑
Hprt1	-	-	-	↑
Ldha	-	-	-	↑

2. Up-regulation of C-C and C-X-C Motif Chemokines

Figure 2. The Sustained up-regulation of the expression of Ccl3, Ccl12, Cxcl2, and Cxcl5 in lung tissues exposed to lunar dust. A. The expression of Ccl3, Ccl12, Cxcl2, and Cxcl5 at high doses (21, and 61mg/m³) of lunar dust (p<0.05 compared to the controls at every time-point); B. The dose-dependent responses of the expression of Ccl12, Ccl3, and Cxcl2 genes in tissues exposed to 0, 2.1, 6.8, 21, and 61mg/m³ doses of lunar dust; C. The dose- and time-dependent responses of the expression of Cxcl5 gene in tissues exposed to 0, 2.1, 6.8, 21, and 60mg/m³ doses of lunar dust.



3. **Correlation between Chemokine Expression and Biomarkers in Bronchoalveolar Lavage (BAL) Cells:** Lunar dust exposure at two highest doses of 21 and 61 mg/m³ induced a significant neutrophil and macrophage infiltration through the mucous membranes of the bronchi.



Correlations between zymosan induced luminol dependent chemiluminescent response and the number of macrophages (A) or neutrophils (B) presented in lavaged fluid from rats exposed to 21mg/m³ (○) or 61mg/m³ (●) dust (13w: red; 4w: blue; 1w: green; and 1d: purple)..

4. Fibrosis related pathways in response to lunar dust induced damage

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value
PPARG	Ligand-dependent nuclear receptor	Inhibited	-2.332	8.70E-12
EGR1	Transcription regulator	Activated	2.575	2.83E-11
NR3C1	Ligand-dependent nuclear receptor	Inhibited	-2.067	6.72E-10
HMG1	Transcription regulator	Activated	2.229	1.56E-09
RELA	Transcription regulator	Activated	2.254	6.54E-09
NR3C2	Ligand-dependent nuclear receptor	Activated	2.200	1.07E-08
KLF2	Transcription regulator	Inhibited	-2.187	9.98E-08

Functions Annotation	p-Value	Predicted Activation State	Activation z-score
Recruitment of Eutrophils	3.95E-14	Increased	2.421
Cell Movement of Neutrophils	7.97E-12	Increased	2.437
Inflammatory Response	8.38E-11	Increased	2.723
Activation of Myeloid Cells	1.64E-10	Increased	2.220
Activation of Neutrophils	2.04E-10	Increased	2.382
Chemotaxis of Monocytes	4.92E-10	Increased	2.164
Activation of Phagocytes	7.38E-10	Increased	2.103
Inflammation of Organ	9.32E-10	Increased	2.161
Adhesion of Monocytes	1.29E-09	Increased	2.169
Adhesion of Phagocytes	3.68E-09	Increased	2.368
Chemotaxis of Neutrophils	1.27E-08	Increased	2.247
Chemotaxis of Myeloid cells	1.51E-08	Increased	2.468
Activation of Leukocytes	2.47E-08	Increased	2.276
Migration of Neutrophils	5.17E-08	Increased	2.221
Degranulation of Phagocytes	2.85E-07	Increased	2.219

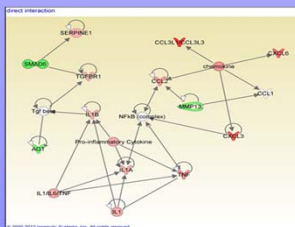


Table 3. (A) List of function s with significant changes in results of lunar dust induced gene expression changes in the lung tissue using the IPA function analysis; (B) List of genes in the direct network interaction and predicted upstream regulators; (C) List of predicted upstream regulators that regulate the gene expression changes in the lung tissue exposed to lunar dust.

CONCLUSION

- Ccl3, Ccl12, Cxcl2, Cxcl5, Itgb8, Tnf, Ldhc, Clec4e, Bmp7, and Smad6, showed persistently significant expression changes in the lung tissue.
- The expression of several of these genes were dose- and time- dependent, and were significantly correlated with other pathological.
- Our previous data showed that no pathological changes were detected in low dose groups. However, several genes, primarily produced by lung epithelial, were significantly altered persistently in response to low-dose dust exposure.
- The data presented in this study, for the first time, explores the molecular mechanisms of lunar dust induced toxicity, contributing not only the risk assessment for future space exploration, but also understandings of the dust-induced toxicity to humans on earth.

ACKNOWLEDGEMENT

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