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Paper

Mechanism of simulated lunar dust-induced lung injury in rats based on transcriptomics

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Lunar dust particles are an environmental threat to lunar astronauts, and inhalation of lunar dust can cause lung damage. The current study explored the mechanism of lunar dust simulant (CLDS-i) inducing inflammatory pulmonary injury. Wistar rats were exposed to CLDS-i for 4 h/d and 7d/week for 4 weeks. Pathological results showed that a large number of inflammatory cells gathered and infiltrated in the lung tissues of the simulated lunar dust group, and the alveolar structures were destroyed. Transcriptome analysis confirmed that CLDS-i was mainly involved in the regulation of activation and differentiation of immune inflammatory cells, activated signaling pathways related to inflammatory diseases, and promoted the occurrence and development of inflammatory injury in the lung. Combined with metabolomics analysis, the results of joint analysis of omics were found that the genes Kmo, Kynu, Nos3, Arg1 and Adh7 were involved in the regulation of amino acid metabolism in rat lung tissues, and these genes might be the key targets for the treatment of amino acid metabolic diseases. In addition, the imbalance of amino acid metabolism might be related to the activation of nuclear factor kappaB (NF-κB) signaling pathway. The results of quantitative real-time polymerase chain reaction and Western blot further confirmed that CLDS-i may promote the occurrence and development of lung inflammation and lead to abnormal amino acid metabolism by activating the B cell activation factor (BAFF)/ B cell activation factor receptor (BAFFR)-mediated NF-κB signaling pathway.

Key words: lunar dust; transcriptome sequencing; differentially expressed genes; pulmonary toxicity mechanism; NF-κB signaling pathway.

Introduction

In recent years, human scientific research on the moon has become the trend and hotspot of deep space exploration in domestic and foreign space activities. Among the harsh and complex environmental factors on the lunar surface, long-term low gravity or microgravity, radiation exposure, and lunar dust and the dust particle environment formed by it still affect astronauts, and solving the impact of lunar dust on lunar exploration activities is one of the problems that must be solved for humans to return to the moon.[1]

It has been reported in the literature that the toxicological effects of lunar dust are highly dependent on the persistence of dust in the lungs, and its persistence is related to particle size, particle morphology, mineral and chemical composition, electromagnetic properties, concentration and exposure time, and cytotoxicity to macrophages.[2] About 20% of the lunar dust particles have a particle size smaller than 20 μm, and 1%–2% of the fine dust particles have a particle size smaller than 3 μm.[3] The main constituents are pyroxene, plagioclase, glass-rich impact particles (cementitic glass), olivine and ilmenite.[4] Studies have shown that inhalation of lunar dust containing olivine, which can precipitate secondary minerals with a fibrous crystal structure that have the potential to induce harmful health effects similar to asbestos exposure.[5] Among the health effects of lunar dust on human body, the biggest threat is its harm to the respiratory system, which mainly comes from the inhalable particles. In particular, particles with a diameter of less than 2 μm can evade the defense mechanism of the upper respiratory tract, reach the lower respiratory tract and alveoli, undergo gas exchange, and then enter the blood circulation, thereby causing damage to the respiratory system and circulatory system, and may even induce cancer.[6] In 1982, some researchers used the tracheal instillation method to change the exposure time of rats in the lunar soil suspension and found that the pathological process of the lungs developed in the form of an inflammatory response.[7] Sun et al.[8] also confirmed this in the acute injury study of simulated lunar dust exposure to rat lung tissue, and their pathological results showed that its pulmonary toxicity was greater than that of PM2.5, and the severity was related to exposure time and dose. However, the underlying mechanism of simulated lunar dust-induced lung injury has not been clearly elucidated.

RNA sequencing (RNA-seq) is one of the high-throughput technologies to comprehensively analyze the transcriptomic changes, and to explore the potential molecular mechanisms through bioinformatics analysis.[9] Transcriptomic technology is widely used in various fields, especially in the study of the toxicological mechanism of PM2.5. The researchers have performed RNA-seq on human umbilical vein endothelial cells

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exposed to PM2.5. There were significant differential expressions of genes involved in stress, inflammation, and vascular dysfunction. Combined pathway analysis showed that these genes were involved in biological processes related to metabolism, inflammation, and host defense against environmental damage. This provides further insights into the underlying mechanisms of PM2.5-induced cardiovascular disease.[10] In addition, studies have shown that lungs exposed to different concentrations of PM2.5 have different transcriptional profiles.[11] As the concentration increased, PM2.5 induced more differentially expressed genes in lung tissue. Studies have results confirmed that gene-specific and genome-wide hypomethylation may be the main mechanism by which PM2.5 exacerbates lung diseases.[12] In addition, Liu et al. also found that PM2.5 exposure induced DNA damage by downregulating the expression of Beas-2B cells (Rad51), disrupting the DNA repair process and leading to genotoxicity of PM2.5.[13]

In previous toxicology studies of simulated lunar dust, the genome-wide transcriptional analysis of lung tissue exposed to simulated lunar dust was not clear, RNA-seq was used to analyze the mRNA expression profile of the transcriptome after exposure, and the biological functions, signaling pathways were analyzed Bioinformatics analysis of protein interaction networks.[9] This will provide us with new insights in the study of the mechanism of simulated lunar dust-induced lung injury. Therefore, this study infected rats with oral and nasal inhalation exposure systems, the lung tissue was removed for transcriptome sequencing. Combined with metabolomics analysis, the toxicity and mechanism of lung injury induced by simulated lunar dust in rats were studied.

Materials and methods

Preparation of simulated lunar dust suspension

The simulated lunar dust sample used in this study is lunar dust simulant (CLDS-i). The CLDS-i is as a gift from the Research Center of Lunar and Planetary Sciences, Institute of Geochemistry, Chinese Academy of Sciences.[14] It contains ~75 vol% glass and a little nanophase metal iron (np-Fe0), and with a median particle size about 500 nm. The CLDS-i particles also have complicated shapes and sharp edges, these properties are similar to those of lunar dust.[15] Store at −80 °C for later use. When used, make up a certain concentration of suspension with normal saline.

Animal grouping and exposure

28 SPF male Wistar rats weighing 190 g ~ 220 g (Beijing Huafukang Biotechnology Co. Ltd, animal license number: SCXK [Jing] 2019-0008) were placed in polycarbonate cages (equipped with HEPA air filter), 2 animals/cage, 12 h–12 h light/dark cycle, room temperature: 24 °C ± 2 °C, relative humidity: 50% ± 5%, free food and water, reared for 1 week, and observed whether their activities and feeding were normal. Then weighed, and randomly divided into 2 groups: a simulated lunar dust-exposed group and a saline-exposed group of 14-rats each.

The Wistar rats in the experimental group were placed in the oral and nasal exposure chamber (Beijing Huironghe Technology Co., Ltd) to be exposed to dust at a concentration of 7 mg/m^3 CLDS-i for 4 h/d, 7 d/week for 4 weeks, and the control group was under the same conditions exposure to saline. Rats were sacrificed one week after the last exposure for the next step of the experiment. Animal experiments were performed in accordance with the guidelines for animal experimentation of Shenyang Medical College, and all animal studies were approved by the Institutional Animal Research Ethics Committee.

Generally

The mental state, fur color, diet, water intake, defecation and urination of the rats were observed every day, and the rats were weighed before, during and after the exposure, and their changes were recorded.

Histopathological examination

The bilateral lung tissues (unwashed) of 4 rats in each group were taken, fixed with 10% paraformaldehyde solution, then dehydrated with ethanol gradient, xylene transparent treatment, lung tissues were embedded in paraffin, and then sliced at 5 μm. Hematoxylin and Eosin (H&E) was used to observe the pathological changes of lung tissue.

RNA sequencing

Part of the lung tissue of the remaining 5 rats in each group was taken, and total RNA was extracted with TRIzol reagent (Invitrogen, CA, USA). Oligo (dT) magnetic beads were used to enrich the mRNA with polyA structure in the total RNA. In this way, the mRNA is broken into fragments of about 300 bp in length. The mRNA is used as a template to synthesize the first strand of cDNA, and the first strand of cDNA is used as a template to synthesize the second strand of cDNA. Then, PCR amplification was used to enrich the library fragments, and then, the quality of the library was checked by the Agilent 2,100 Bioanalyzer, and then the total concentration of the library and the effective concentration of the library were detected. Libraries containing different Index sequences (adding different Index to each sample, and finally distinguishing the offline data of each sample according to the Index) were mixed in proportion. The pooled library was uniformly diluted to 2 nM and denatured by alkali to form a single-stranded library. After RNA extraction, purification, and library construction of the samples, the next-generation sequencing technology was used to perform paired-end sequencing on these libraries based on the Illumina sequencing platform.

Transcriptome bioinformatics analysis

First, the raw data (Raw Data) were filtered, and the high-quality sequences (Clean Data) obtained after filtering were aligned to the reference genome of the species. According to the comparison results, the expression level of each gene was calculated. On this basis, we used DESeq to perform differential analysis of gene expression, and the conditions for screening differentially expressed genes were: expression difference fold |log2FoldChange| > 1, significant P-value<0.05, significant P-value<0.05. The differential genes were visualized by volcano plot and cluster analysis. Then, Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) functional annotation and enrichment analysis were performed on the differential genes. GO annotation and enrichment analysis were applied to evaluate the biological function of differentially expressed genes (DEGs) and distribution in GO categories. KEGG pathway annotation and enrichment analysis were conducted to screen the most important signal transduction pathways involved in DEGs.

Metabolomics research

Lung tissues of rats treated with saline (n = 5) or CLDS-i (n = 5) were collected. After homogenizing, and disposing in 400 μL of pre-cooled methanol/acetonitrile/water solution (4:4:2, v/v) at −20 °C for 60 min, the samples were centrifuged at 14,000 g for 20 min at 4 °C. The supernatant was collected and vacuum

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