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An acetyl-L-carnitine switch on mitochondrial dysfunction and rescue in the metabolomics study on aluminum oxide nanoparticles

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Abstract

Background: Due to the wide application of engineered aluminum oxide nanoparticles and increased aluminum containing particulate matter suspending in air, exposure of human to nano-scale aluminum oxide nanoparticles (Al_2O_3 NPs) is becoming inevitable.

Methods: In the present study, RNA microarray coupled with metabolomics analysis were used to uncover mechanisms underlying cellular responses to Al_2O_3 NPs and imply the potential rescue.

Results: We found that Al_2O_3 NPs significantly triggered down-regulation of mitochondria-related genes located in complex I, IV and V, which were involved in oxidative phosphorylation and neural degeneration pathways, in human bronchial epithelial (HBE) cells. Subsequent cell- and animal- based assays confirmed that Al_2O_3 NPs caused mitochondria-dependent apoptosis and oxidative stress either in vitro or in vivo, which were consistent with the trends of gene regulation. To rescue the Al_2O_3 NPs induced mitochondria dysfunction, disruption of small molecular metabolites of HBE were profiled using metabolomics analysis, which facilitates identification of potential antagonizer or supplement against nanoparticle-involved damages. Supplementation of an antioxidant, acetyl-L-carnitine, completely or partially restored the Al_2O_3 NPs modulated gene expression levels in mitochondrial complex I, IV and V. It further reduced apoptosis and oxidative damages in both Al_2O_3 NPs treated HBE cells and animal lung tissues.

Conclusion: Thus, our results demonstrate the potential mechanism of respiratory system damages induced by Al_2O_3 NPs. Meanwhile, based on the metabolomics profiling, application of acetyl-L-carnitine is suggested to ameliorate mitochondria dysfunction associated with Al_2O_3 NPs.

Keywords: Aluminum oxide nanoparticles, Mitochondria, Acetyl-L-carnitine, Nanotoxicology, Metabolomics

Background

Nano-scale aluminum oxide (Al_2O_3) particles are widely used in insulator layers, powder coating, and fluorescent lamp material. Meanwhile, aluminum (Al) is a common metal component in ultrafine airborne particles ($PM_{2.5}$) in the ambient environment[1,2] and is relatively stable in the form of aluminum oxide. These ultrafine aluminum particles have different physical, chemical, and biological characteristics, and are likely to be more toxic than the same conventional sized materials [1]. Thus, increased and inevitable occupational and environmental exposures to ultrafine Al_2O_3 particles are considered as a health risk, while, prevention or intervention measures have not been investigated. Generally, ultrafine particles can be inhaled more deeply than large particles, leaving sediment on the surface of the trachea, bronchus, and alveoli. Lung tissue is considered the primary target organ

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for inhaled ultrafine particles. Accordingly, aluminum oxide nanoparticles (Al_2O_3 NPs) were employed as an ultrafine particle model to explore the impacts of aluminum ultrafine particles on the respiratory system.

Apoptosis is an endpoint to assess toxicity of metal oxide nanomaterial, providing a benchmark against which to evaluate the potential toxicity of engineered nanoparticles [2–4]. There are two major apoptotic pathways initiated by either mitochondria or cell surface receptors [5]. Mitochondria-mediated apoptosis is also called the 'intrinsic' pathway and occurs in response to a wide range of death stimuli, and is characterized by cascade reactions including mitochondrial outer membrane permeabilization (MOMP), release of cytochrome *c* into the cytoplasm to activate caspase-9 and subsequent caspase-3 [5, 6]. The intrinsic pathway is involved in immune disorders [7], neurodegeneration [8] and cancer [9, 10]. Studies investigating ambient particulate matter or nanoparticles suggest apoptosis or mitochondrial dysfunction are sufficient end-points to monitor toxicity [11–13], but conventional toxicity assays may not suffice to fully understand the cellular responses of ultrafine particle exposure. Thus, a more comprehensive approach to determining how cells respond to ultrafine particles is required.

"Omics" analysis, including transcriptomics, proteomics, and metabolomics, coupled with appropriate computational approaches to determine statistically significant gene, protein, metabolite or pathway regulation can be used as a tool to identify the potential hazards and mechanisms of nanoparticle toxicology [14–16]. These newly developed high-throughput approaches have been used to study the impact of nanomaterials, including metal and metal oxide nanoparticles [17–19]. In such studies, the "omics" technologies are used to predict interaction between nanoparticles and biological systems, facilitate assessment of systemic toxicity due to nanomaterials, reveal potential strategies for risk mitigation.

Metabolomics analysis is one of the most applied "omics" analysis that facilitates understanding of the modulation of small molecules following exposure. Acetyl-L-carnitine (ALCAR), an antioxidant dietary supplement, could be detected through GC/TOF/MS analysis and plays a vital role in oxidation of fatty acid metabolic pathways. It is a constituent of the inner mitochondrial membrane and has many fundamental functions, including acetyl CoA uptake [20], improving mitochondrial bioenergetics [21] and prevention of mitochondrial enzyme oxidation [22]. The acetyl group of ALCAR is used to produce the antioxidant glutathione (GSH), reducing oxidative stress, and protecting cells against lipid peroxidation [23, 24]. ALCAR also contributes to the bioenergetics processes, therefore, it plays a vital role in mitochondrial-related disorders [25, 26].

Here, we used human bronchial epithelia (HBE) as a model system due to their importance in defense against inhaled pathogens and particulates [27]. We investigated changes in gene expression profiles and small molecular metabolites in HBE cells induced by Al_2O_3 NPs. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to evaluate pathways. We showed that Al_2O_3 NPs are capable of triggering specific changes in HBE cell gene expression, specifically in mitochondria associated genes. We assessed mitochondria-mediated apoptosis in the presence or absence of acetyl-L-carnitine as a supplement to oppose mitochondrial dysfunction caused by Al_2O_3 NPs. The pathological alterations and ALCAR rescue were observed in mouse lung tissues treated with Al_2O_3 NPs. Our results suggests Al_2O_3 NPs cause toxicity and mitochondrial dysfunction that may be remedied by acetyl-L-carnitine treatment.

Results

Overview of mRNA microarray profiles

To determine how gene transcription was altered due to Al_2O_3 NP exposure, HBE cell mRNA profiling was evaluated by RNA microarray. As shown in Fig. 1a, Al_2O_3 nanoparticles exerted specific transcriptional effects leading to significantly increased expression of 54 genes and decreased expression of 304 genes, with a cutoff as 2 or more fold change and $P < 0.05$. GO enrichment classifies transcriptional changes observed between control and treatment groups. Notably, the GO analysis revealed that genes encoding proteins necessary for mitochondrial function were differentially expressed. Significantly, of the genes differentially regulated, 23/154 genes involved in cell components (CC) (Fig. 1b); 11/85 genes involved in molecular function (MF) (Fig. 1c), and 14/183 in biological process (Fig. 1d). These mitochondria involved enrichment were labeled with red color in Fig. 1. Therefore, a total of 27 mitochondrial related genes were identified, the fold change, *P* value and functions of their encoded proteins are shown in Table 1.

Subsequently, these 27 genes were subjected to KEGG pathway enrichment analysis. These selected genes were annotated with the KEGG pathway database and hypergeometric tests were used to estimate the significance of enrichment. Among these pathways, mitochondrial function and neural system disease were significantly enriched (Fig. 2a). Figure 2b is schematic oxidative phosphorylation pathway by KEGG. Our microarray data suggested that NADH ubiquinone oxidoreductase-encoding genes (ND UFA2 and NDUFS4), NADH dehydrogenase-encoding genes located in mitochondrial complex 1 (NDUFC2, NDUFA1, and NDUFA4), NADH ubiquinol-cytochrome *c* reductase gene UQCR11 (complex III), cytochrome *c* oxidase genes (COX7B and COX17 (complex IV)), and the

Methods

Nanomaterials and animals

Al_2O_3 NPs were purchased from Plasmachem GmbH, Germany (>99.8 % purity). The particle size and zeta potential of Al_2O_3 NPs in PBS suspension was 64.17 nm and 37.1 mV, which was analyzed by a zetasizer (nanozs90, Malvern Instruments, UK) (Additional file 1: Figure S3).

Thirty-six male and 36 female ICR mice (20–22 g) were purchased from Shanghai SLRC Laboratory Animal Co. Ltd., China.

Cell culture and RNA extraction

The human bronchial epithelial cell line (HBE) (American Type Culture Collection) was maintained in Dulbecco's modified Eagle's medium (DMEM) at 37 °C in 5 % CO_2. The culture medium was supplemented with 10 % (v/v) fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were seeded in 10 cm culture dishes and exposed to 100 μg/ml Al_2O_3 NPs with three biological replicates. At 24 h post treatment, complete medium was removed and adherent cells were collected. Total RNA was extracted using the TRIZOL reagent (Invitrogen, U.S.) according to the manufacturer's instructions.

RNA microarray and gene expression analysis

An Agilent Array platform (Agilent Technologies, Santa Clara, CA, USA) was employed for microarray analysis. The labeled cRNAs were hybridized onto a Human LncRNA Array v3.0 (8 × 60 K; arraystar) chip designed for 26 109 coding genes. The arrays were scanned using an Agilent G2505C scanner and the fluorescence intensity was analyzed with Agilent Feature Extraction software (version 11.0.1.1). Quantile normalization and subsequent data processing were performed using the GeneSpring Gx v12.0 software package (Agilent Technologies). An absolute fold change of 2 or more and 0.05 adjusted P-value were set as cutoff to evaluate the significance of gene expression differences of raw data.

Functional group analysis

DAVID 6.7 (Database for Annotation, Visualization, and Integrated Discovery), a functional annotation tool, was used to analyze differentially expressed genes. The DAVID functional annotation cluster tool provides three structured networks of defined terms that describe the attributes of gene products. The P-value was set to 0.1 to denote the significance of GO enrichment in the differentially expressed mRNA list. Fold enrichment (-lg (*P*-value)) was used to denote the enrichment of a particular GO term. Pathway analysis for differentially expressed mRNAs was performed on the KEGG database. This analysis determines the biological pathways for which a significant enrichment of differentially expressed mRNAs exist (*P*-value was set as 0.05).

Metabolomics analysis of Al_2O_3 NPs treated HBE cells

GC/TOFMS analysis was performed using an Agilent 7890 gas chromatography system coupled with a Pegasus 4D time-of-flight mass spectrometer. HBE cells were exposed to Al_2O_3 NPs (0, 100, and 500 μg/ml) for 24 h with 9 biological replicates. Then the cell lysate sample were prepared and analyzed as described [16].

Animal treatment

Mice were maintained and used according to the guidelines of the Committee on Animal Use and Care of Southeast University. The dynamic inhalation exposure chambers were outfitted with an aerosol generator (Beijing HuiRongHe Technology Co., Ltd, China). . Mice were divided into six groups (with six male and six female mice in each group): control, mice treated with 100 mg/kg ALCAR, mice treated with low and high doses of Al_2O_3 NPs, mice treated with a low dose of Al_2O_3 NPs + 100 mg/kg ALCAR, and mice treated with a high dose of Al_2O_3 NPs + 100 mg/kg ALCAR. Mice were housed six per polycarbonate cage on corncob bedding with ad libitum access to food and water. Exposure was carried out in three whole-body inhalation chambers; two chambers received low or high dose of Al_2O_3 NPs and the control chamber received HEPA-filtered clean air at the same flow rates as the experimental groups. Six males and six females were exposed in each chamber for 8 h per day for 7 consecutive days. Light cycles were set on a 12/12 h light/dark cycle. Al_2O_3 NPs in the chamber were generated by the aerosol generator and mean concentrations of Al_2O_3 NPs were 2 mg/m^3 and 10 mg/m^3 for low and high dose treatments, respectively. The concentration of Al_2O3 NPs were monitored by an extensive air quality monitoring equipment (CEL-712 Microdust Pro, CASELLA, UK). 100 mg/kg ALCAR was orally administrated by gavage. The temperature, air flow rate and relative humidity in the chambers was set to 22.5 °C, 40 L/min, and 50 ± 10 %.

Histopathological analysis of mice lung tissue

Mice were euthanized under ether anesthesia 1 h after the end of dynamic Al_2O_3 NP exposure on the 7th day. All the mice were decaptitated on an iced table. Lung tissue was divied for three parts. One piece was immediately prepared for capasse activity, ATP level, ROS and MDA analysis. The second piece was stored in liquid nitrogen, while another piece was preserved in 4 % paraformaldehyde (PFA) for 24 h at 4 °C, embedded in paraffin, serially sectioned (5 μm) and mounted on silane-covered slides. After dewaxing, the sections selected from