Proteomic analysis of proteins from bronchoalveolar lavage fluid reveals the action mechanism of ultrafine carbon black-induced lung injury in mice
Chih-Ching Chang¹, Shu-Hui Chen², Shih-Hsin Ho², Chun-Yuh Yang³, Hong-Da Wang⁴, Mei-Ling Tsai⁴,*

¹Department of Environmental and Occupational Health, National Cheng Kung University, Tainan, Taiwan
²Department of Chemistry, National Cheng Kung University, Tainan, Taiwan
³Graduate Institute of Public Health, Kaohsiung Medical University, Kaohsiung, Taiwan
⁴Department of Physiology, National Cheng Kung University, Tainan, Taiwan
mltsai@mail.ncku.edu.tw

Proteomics 2007; 7:4388-4397

Recently, there are rapid increases of airborne and engineered nanoparticles (≦100 nm in diameter; Smaller than bacteria and epithelial cells). This is due to the strict environmental protection regulations and the development of nanotechnology. Environmental protection agency (EPA) around the world regulates industrial or vehicular emissions on mass output. To meet the regulations, new internal combustion engines are designed to generate more nanoparticles because nanoparticles contributes very little to the mass output. On the highway, nanoparticles can reach 1x10⁷ particles per cm³ of air. Epidemiologic studies have demonstrated that nanoparticles play a more significant role in producing respiratory disease and detrimental respiratory effects than larger particles. At the same time, engineered nano-structured materials are rapidly developed by material scientists. For example, ultrafine titanium dioxide (ufTiO₂) serves as photocatalyst, capable of generating oxygen radicals to kill pathogens. Carbon nanotubes are one of the most attractive nanomaterials. They are lightweight materials with strong tensile strength and can conduct electricity better than copper. So, they have wide applications in electric and aerospace industry. With the increased production and use of these new nanomaterials, this has created great concern on their possible health effects.

Nanoparticles have the greatest particle number, surface area and surface molecules per unit of deposited particle mass, compared with other larger particles. These properties enable nanoparticles to escape phagocytosis by macrophage and induce oxidative stress of target cells. In vivo studies have found that nanoparticles provoke influxes of polymorphonuclear cells, enhance protein exudation into alveolar space, prolong particle interstitial retention, and increase lung lymph node burden to a greater extent than fine particles in rats. After inhaled into the lung, nanoparticles can easily translocate to heart, liver, brain and kidney within 30 min. They can cause reduced heart rate variability and enhanced thrombosis. Our previous study has shown that nanoparticle can act through a ROS-dependent pathway to cause pulmonary edema, through the production of vascular endothelial growth factor (VEGF), and lung injury in mice (1).
As for carbon nanotubes, it is found to induce pulmonary interstitial inflammation and granulomatous/fibrotic changes in mice. These histopathological findings are similar to those found in asbestos-exposed animals and human.

Proteomic studies allow qualitative identification or quantitative comparison of proteins from biological samples of particular disease stage. It is a descriptive study and offers a powerful alternative first-step to hypothesis generation. In order to understand more on the mechanisms involved in the pathogenesis of nanoparticle-induced lung injury, we take advantage of proteomic analysis to establish the proteome of bronchoalveolar fluid (BALF) by 1-D gECL/MS/MS in the mice exposed to nanoparticle (Ultrafine carbon black, 14 nm in diameter) (2). The use of 1-D gECL/MS/MS increases the chance of identifying high molecular weight proteins, low abundant cellular receptor and poorly water-soluble membrane and nuclear proteins, which are frequently missed in conventional 2-D MALDI-MS analysis. A total of thirty-three proteins are identified, including three membrane proteins [leukemia inhibitor factor receptor (LIFR), epidermal growth factor receptor (EGFR) and SEC14-like 3] and one high molecular weight protein [protease inhibitor α2M] (Figure 1). In the validation study, western blot analysis find that LIFR, EGFR, anti-oxidant ceruloplasmin and α2M are significantly increased in BALF of ufCB group. Furthermore, ufCB exposure reduces the protein abundances of LIFR and EGFR in the lung tissue (Figure 2). Nanoparticle exposure also increases the production of ceruloplasmin from pulmonary epithelial cells, indicating increased oxidative stress after exposure (Figure 3). Taken together, we propose that acute exposure to ufCB caused oxidative stress and protease release, resulting in epithelial shedding with significantly increased LIFR and EGFR in BALF.

Moreover, nanoparticle exposure increases the production of LIF in BAL fluid and decreases the expression of LIFR in pulmonary epithelial cells. Conceivably, nanoparticle exposure can activate LIF/LIFR pathway. LIF is a member of the interleukin-6 superfamily. LIF exaggerates inflammatory responses by inducing
leukocyte differentiation. It can influence airway inflammation by modulating the expressions of tachykinin and its receptor. Importantly, LIFR is also present on cholinergic nerve and excitatory non-cholinergic non-adrenergic (eNANC) sensory nerves of airways. Via its receptor, LIF can upregulate the contractile effects of sensory nerves and cholinergic nerves on smooth muscle. As a result, LIF may provide a bi-directional cross-talk between immune systems and neural tissues in inflammation insult. However, little is known about LIF in the context of nanoparticles-induced pulmonary inflammation and adverse respiratory effects. Currently, our lab is investigating the role of LIF and its interactions with neural systems in the development of adverse respiratory outcome after nanoparticle exposure.

References:

1. Chang CC, Chiu HF, Wu YS, Li YC, Tsai ML, Shen CK, Yang CY. The induction of vascular endothelial growth factor by ultrafine carbon black contributes to the increase of alveolar-capillary permeability. Environ Health Perspect 2005; 113, 454-460