学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Molecular responses of human lung epithelial cells to the toxicity of metal oxide nanoparticles

（金属酸化物ナノ粒子に対するヒト肺上皮細胞の毒性応答分子機構の解明）

Nowadays, human exposure to the engineered nanoparticles has become inevitable since nanoparticles are widely used in various industries and products. Among the nanomaterials, metal oxide nanoparticles are widely applied because of their unique properties, but the toxicity of them has also been reported by both *in vitro* and *in vivo* assessments. To clarify recent controversial reports on the mechanism of zinc oxide nanoparticles (ZnO-NPs) cytotoxicity and the dramatic cytotoxicity of copper oxide nanoparticles (CuO-NPs), we focused on the study on the molecular mechanism of human lung epithelial cells (A549 cells) in response of exposure to them. By analyzing the global gene expression with micro array technology, we proved the ROS was critical not only in toxicity of the transient metal oxide nanoparticles (CuO-NPs) but also in the toxicity of semiconductor metal oxide nanoparticles (ZnO-NPs). Furthermore, we discovered that CuO-NPs and ZnO-NPs produced ROS and killed the cells in the different ways.

In chapter 1, general information on the nanomaterials and the nanotoxicity was introduced to clarify the concepts in present study. Also, the origin of the healthy risk concern about the nanomaterials and the new anxiety raised by the nanotechnology were combed to explain the necessity of our study. The development of nanotoxicity research was summarized and the discrepancies of these studies on nanotoxicity were discussed to explain the object of the present study.

In chapter 2, to clarify the controversial report on the cytotoxicity of ZnO-NPs, we tried to identify the respective contributions of the released zinc and the solid particles to the cytotoxicity of ZnO-NPs, we exposed A549 cells to the ZnO-NPs suspensions, their extractions collected with centrifugation and the medium containing zinc chloride ($ZnCl_2$), then we assayed the cytotoxicity with water soluble tetrazolium salts (WSTs) and the intracellular reactive oxygen species (ROS) with the 2`,7`- dichlorodihydrofluorescin diacetate (DCFH-DA). Only the ZnO-NPs suspension brought about the cytotoxicity and the increase of intracellular ROS; the extractions and $ZnCl_2$ had no effect on the cells. However the subsequent global gene expression analysis revealed that “cadmium binding category” was the only one gene functional category which was up-regulated not only by $ZnCl_2$ medium also by ZnO-NPs suspension. This category consisted of metallothioneins (MTs), the important zinc-toxicity neutralizing proteins. We inhibited the over expression of MTs with the corresponding siRNA, and found that the released zinc contributed to the ZnO-NPs cytotoxicity. We conclude that both of the solid particles and the released zinc contribute to the ZnO-NPs cytotoxicity, further we propose the synergic relationship of them through disabling the MTs via ROS.

In chapter 3, the toxicity mechanism of CuO-NPs was discussed with global gene expression analysis because CuO-NPs were proved to be the most toxic nanoparticles among the most widely used nanomaterials. With the same analysis methods, this study proposes a molecular mechanism for lung epithelial A549 cell response to CuO-NPs related to Cu ions released from CuO-NPs with the same analysis methods as in previous ZnO-NPs research. Cells that survived the exposure to CuO-NPs arrested the cell cycle as a result of the down-regulation of proliferating cell nuclear antigen (PCNA), cell division control 2 (CDC2), cyclin B1 (CCNB1), target protein for Xklp2 (TPX2), and aurora kinase A (AURKA) and B (AURKB). Furthermore, cell death was avoided through the induced expression of nuclear receptors (NR4A1 and NR4A3) and growth arrest and DNA damage-inducible 45 β and γ (GADD45B and GADD45G, respectively). The down-regulation of CDC2, CCNB1, TPX2, AURKA, and AURKB, the expressions of which are involved in cell cycle arrest, was attributed to Cu ions released from CuO-NPs into medium. NR4A1 and NR4A3 expression was also induced by Cu ions released into the medium. The expression of GADD45B and GADD45G activated the p38 pathway that was involved in escape from cell death. The upregulation of GADD45B and GADD45G was not observed with Cu ions released into medium but was observed in cells exposed to CuO-NPs. However, because the expression of the genes was also induced by Cu ion concentrations higher than that released from CuO-NPs into the medium, the expression appeared to be triggered by Cu ions released from CuO-NPs taken up into cells. We inferred that, for cells exposed to CuO-NPs, those able to make such a molecular response survived and those unable to do so eventually died.

In chapter 4, the results of present studies were summarized, the contribution of ROS was discussed respectively in the case of transient metal oxide nanoparticles and the in the case of the semiconductor metal oxide. On the basis of the global gene expression analysis, we could not only have an overall view of the cellular response to the sudden exposure to nanoparticles, we also could explore the unknown key toxicant exceeding what we have expected. Thus, we propose the importance of global gene expression analysis in the future explore in nanoparticles. Additionally, on the basis of the present study we also come to cognized that once the nanoparticles were dispersed into biological environment all the chemicophysical characterizations changed immediately, especially the interface chemical, which is very important to the final bio-toxicity, we think the veil of nanotoxicity of the engineered nanomaterials will be uncovered when this interface chemical is studied fully.