Development of the methodology of searching for new biomarker of carcinogenesis and application to elucidation of the genotoxicity mechanism induced by nanomaterials

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In recent years, nanomaterials are commonly used in various fields such as cosmetic, medical and so on. However, the genotoxicity of nanomaterials have been not fully elucidated yet. In this study, we investigated the detailed induction mechanism of genotoxicity of nanomaterials using cultured mammalian cells.

We assumed that the genotoxic effects of nanomaterial may be caused by the incorporation of them into cells. So, we performed the flow cytometric (FCM) analysis to confirm the incorporation of Kaolin, which showed relatively-strong genotoxicity in the lungs of mouse, into alveolar epithelial cells (A549) and macrophage cells (RAW264). Kaolin was incorporated into both A549 and RAW264. Especially, RAW264 incorporated more kaolin compared to A549. At this time, we investigated the generation of reactive oxygen species (ROS) from these cells using FCM with DCFH-DA fluorescein. The numbers of ROS generated from RAW264 was increased with incorporation of kaolin, whereas the numbers of ROS from A549 was not increased. To examine the relationship between parenchymal and macrophage cells exposed with Kaolin, we developed the *in vivo* mimic system, co-culture with A549 and RAW264. When only RAW264 was exposed with kaolin in this system, the number of ROS generated from A549 cells was increased and DNA damage was induced in A549 cells. Additionally, we performed the comet assay with Fpg protein treatment to estimate the induction of oxidative DNA damage, such as 8-oxodG, in A549 cells. As a result, DNA damage induced by Kaolin was contributed by oxidative stress.

These results suggest that the genotoxicity of Kaolin may be induced by ROS and/or other factors, such as TNF-α and IL-1β, released from macrophages incorporating kaolin. It is expected that the detailed induction mechanisms of oxidative DNA damage will become clear by the analysis of other factors.