Title of Research:

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Construction of novel *in vitro* evaluation systems based on genotoxic mechanisms of nanomaterials

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Collaborators:

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Summary of Research: ***To establish new in vitro evaluation systems for lung/skin toxicity of nanomaterials, we aimed to establish assay models as listed below.***

① A novel in vitro genotoxicity assay model to assess lung toxicity using a co-culture system.

② A novel in vitro assay model to assess skin toxicity using 3D human skin reconstitution models.

③ A novel in vitro assay model using 3D culture techniques such as tissue-slice and spheroids.

For the lung toxicity test, we established a co-culture system of GDL1 and RAW264 cells. We evaluated nanomaterials having differences in physicochemical character by using this co-culture system. In the present study, we chose multi-walled carbon nanotubes (MWCNTs) with different sizes, and magnetite (MGT) with/without surface modification as test nanomaterials. When gpt delta mice were intratracheally instilled with multiple doses of different sizes of MWCNTs, mutation frequency (MF) observed in the lungs was higher than that of the vehicle control. However, the influence of size differences against MF was not observed in the lungs of mice exposed to these MWCNTs. Supporting this, a similar result was observed in the co-culture assay system. We also evaluated MF induced by MGT with/without surface modification using the co-culture system, and there was a tendency that higher MF was observed in the cells exposed to MGT with surface modification than that of MGT without surface modification. Therefore, it is suggested that the co-culture

assay model can be considered as a suitable evaluation system for nanomaterial toxicity for the lungs.

As a 3D skin model, we selected the LabCyte EPI model. Using biochemical and histopathological techniques, we assessed cytotoxicity and ability of gold and silver nanoparticles to invade into the skin using a reconstituted 3D human skin model (LabCyte EPI model, Japan Tissue Engineering Co., Ltd.) and HepG2 cells. In the reconstituted 3D human skin model, neither gold nor silver nanoparticles killed or invaded into the epidermis up to 1000 μg/mL, while neither nanoparticle killed HepG2 cells up to 100 μg/mL. It is thus suggested that gold or silver nanoparticles do not possess cytotoxicity or ability to invade into the skin, at least under the present experimental conditions. Furthermore, the LabCyte EPI model may be useful as a novel in vitro system to assess percutaneous toxicity of nanomaterials.

On the other hand, since the respiratory system is susceptible to damage resulting from inhalation of nanomaterials, the assay of A549 spheroids layered on histological sections was used for MGT induced cytotoxicity. Results of spheroid disruption by cellular adhesion damage and cell viability by Alamar Blue assay showed the possibility of the assay of A549 spheroids layered on histological sections as a toxicological safety test.

Timeline: March 1st, 2016 – February 29th, 2017

Topics: “Construction of novel in vitro evaluation systems based on genotoxic mechanisms of nanomaterials “ Presented at the poster session of the Annual Conference of New JCIA-LRI

Publications:

Journals:

1. KatoT, Toyooka T, Ibuki Y, Masuda S, Watanabe M, Totsuka Y. Effect of Physicochemical Character Differences on the Genotoxic Potency of Kaolin. Genes Environ., in press.
2. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo, “Theranostic Nanoparticles for MRI-Guided Thermochemotherapy: Tight Clustering of Magnetic Nanoparticles Boosts Relaxivity and Heat-Generation Power” ACS Biomaterials Science & Engineering, 3, 95–105, 2017.

Meetings:

1. TotsukaY, Watanabe M, Hayashi K, Nakae D: Development of a novel in vitro mechanism-based evaluation system of the genotoxicity of nanomaterials 45th EEMGS(Copenhagen、Aug, 2016)
2. Sato H, Sakamoto Y, Nakae D, Totsuka Y: Effect of Physicochemical Character Differences on genotoxic potency of MWCNTs 45th JEMS (Tsukuba、Nov, 2016)