Elucidation of dynamic behavior of exosome membranes involved in intracellular uptake efficiency

([1]*Graduate School of Science, Osaka University*, [2]*Institute for Glyco-core Research (iGCORE), Gifu University*) ○ Tomokazu Yasuda,[1] Koichiro M. Hirosawa,[2] Kenichi G.N. Suzuki,[2] Kabayama Kazuya, [1] Michio Murata,[1] Shinya Hanashima[1]

Keywords: exosome, fluorescence spectroscopy, membrane fluidity, intracellular uptake, fluorescent microscopy

Exosomes, a type of extracellular vesicles (EVs), are small vesicles with a diameter of 40-150 nm. The exosomes regulate biological processes by transporting proteins and nucleic acids as an intracellular communication tool. Through the recent lipidomics studies [1], exosome membranes are found to be often enriched with sphingomyelin and cholesterol (Cho), which are typical lipids for the microdomains (lipid rafts) in the plasma membrane. However, the membrane dynamics of exosomes involved in regulating the activity of functional biomolecules have hardly been elucidated.

We evaluated the membrane dynamics of exosomes by fluorescent spectroscopy (anisotropy and lifetime) and compared them with the artificial model membranes. The results suggested the presence of raft-like ordered domains, and the different dynamics between outer and inner leaflets in a similar manner to those of plasma membranes. In addition, these lateral and interleaflet heterogeneities of exosome membranes were variable depending on the exosome-derived donor cell lines.

Moreover, we artificially prepared Cho-poor exosomes using methyl-β-cyclodextrin, and observed the uptake of the exosomes into the cells with fluorescence microscopy. By comparing with the uptake of the intact exosomes, we were able to clarify the importance of the exosomal Cho in the cellular uptakes of exosomes.

[1] Skotland, T.; Sandvig, K.; Llorente, A. *Prog. Lipid Res.* **2017**, *66*, 30-41.