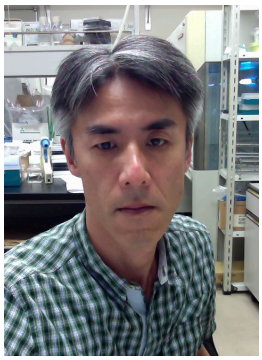
Principal Investigator: Hiromu Takematsu
Grant Title: Molecular mechanisms of cytokinetic inhibition triggered by psychosine

Abstract



Psyochosine is a lyso-form of galactosylceramide and psychosine accumulation is reported in the genetic loss of galactosylceramidase in Krabbe disease. Psychosine seemed to account for the neural toxicity found in patients and we have been studying the molecular mechanism of psychosine-mediated globoid cell formation, which is used as a diagnosis of the patient. Globoid cells are giant multiploid cells and we found that psychosine could inhibit cytokinesis, a last step in mitosis to produce such giant cells. In this study, the molecular mechanism how psychosine could turn mitotic cells to form multiploid cells was investigated. This was of particular interest because psychosine-mediated multiploid cell formation could be classified as “endomitosis”, a specific type of mitosis. In endomitotic cell division, cells keep cell cycle to duplicate cellular contents whereas division part is disabled. In fact, endomitosis is indispensable for the formation of mature megakaryocyte, which is precursor cell type of platelet formation. To date, it is not known how endomitotic cell cycle and normal mitosis is regulated.

In this study, a search for receptor molecule of psychosine was conducted. However, such attempt failed. Instead, we found that membrane cluster sphingomyelin (SM), localized in outer leaflet of cellular membrane, was disrupted by psychosine. In collaboration with Dr. Toshihide Kobayashi, a loss of lysenin-probe signal was detected, which is specific for the cluster SM. This was not a loss of SM from membrane because another probe, equinatoxin signal was not affected by the psychosine. Loss of SM cluster seemed to cause loss of phosphatidylinositol-4, 5 kinase enzyme from the site and consequential loss of phosphatidylinositol 4, 5 bisphosphate production as probed by the use of PH-GFP probe. This loss was found in the cleavage furrow, a regression of membrane in the site of cell division, more specifically at the cytokinetic stage or telophase of mitosis.

Psychosine seemed to mimic membrane glycosphingolipid (GSL) because abundance of GSL could enhance psychosine function. Interestingly, abundance of SM reduced psychosine function to induce cellular endomitosis. These data indicated that membrane balance between SM and GFL functions against each other. From these experimental results, we concluded that psychosine could target cellular membrane domain, more specifically, cluster SM to switch mitotic cells to endomitotic cells.

Psychosine-sensitive cells were utilized to understand downstream effector molecules. Although we were able to isolated psychosine-resistant cell clones as proposed, however, targeted molecules downstream of psychosine have not identified yet. Progress in this set of experiments are expected in the future study.