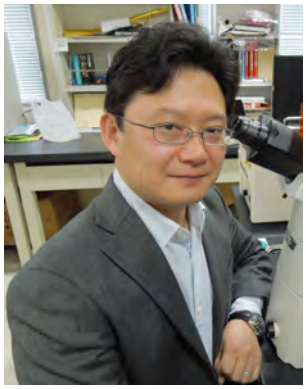
Principal Investigator: Kazuaki Ohtsubo
Grant Title: Establishing novel paradigm of glycan-mediated regulation of glucose homeostasis

(a) Abstract

1. *Objectives*



We have previously reported that cell surface expression of glucose transporter (GLUT) is sustained by binding with Galectins using its N-glycan in pancreatic β cells. Disruption of the Galectin binding induces the translocation of GLUT from non-lipid raft micro-domain to lipid raft micro-domain on cell surface that is followed by internalization of GLUT. This reduces the cell surface residency of GLUT and impairs glucose-stimulated insulin secretion that results in the failure of pancreatic beta cells observed in the early stage of type 2 diabetes. Although the biological significance of the formation of Galectin complex has been well documented, the detailed mechanism of the retention of GLUT in non-lipid raft microdomain has not been well elucidated. In the present study, we analyzed the membrane microdomain distribution and the constituent molecules of the Galectin complex to better understand the micro-mechanism regulating functions and activities of cell surface glycoproteins that should contribute to the elucidation of the glycan-mediated general regulation mechanism in glucose homeostasis.

2. *Methods*

The membrane microdomain distribution of Galectins was determined by immunoblot analyses conjunction with sucrose density gradient centrifugation of plasma membranes prepared from pancreatic β cells. The constituent molecules of the cell-surface Galectin-complexes were indentified by the immunoprecipitation-based isolation of the complexes and Mass spectrometry.

3. *Results*

The results of these experiments showed that Galectins are distributed in non-lipid raft microdomains. This was in agreement with the cellular distribution of GLUT. The constituent molecules of the cell-surface Galectin-complexes were identifies as cationic amino acid transporter-3 (CAT3), Teneurin-3, Anionic trypsin-2, Myosin-4, Actin, α-tubulin, GLUT2. These are well characterized as functional molecules, sensing the concentration of nutrition and stimulating pancreatic β cells to secrete insulin, remodeling protein complexes, anchoring proteins, and sustaining cellular architectures. These findings suggested that the Galectin is a key molecule in the regulation of distribution and function of cell surface glycoproteins.