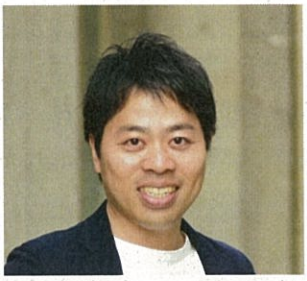
SAMPLE OF THE ABSTRACT

Principal Investigator: Jun Suzuki

Grant Title: Identification and functional analysis of glucosylceramide scramblase

Abstract

Purpose



In mammalian cells, ceramide (Cer) is generated at Endoplasmic Reticulum, and transferred to Golgi Apparatus. Glucose is added to Cer by UGCG at the cytoplasmic side of Golgi to generate glucosylceramide (GlcCer). The synthesized GlcCer is then scrambled into the luminal side of Golgi Apparatus where galactose is added by β4Galt5 to generate lactosylceramide (LacCer). The generated LacCer is further modified by sugars to generate varieties of gangliosides or directly transferred to the plasma membranes where it functions as a platform for the signaling complex. Although molecular mechanisms of glycolipids synthesis are well understood, it has been unknown how the synthesized GlCer is scrambled into the luminal side of Golgi Apparatus. Here, we tried to reveal molecular mechanisms of GlCer scrambling using unbiased screening approaches.

Methods

We searched for cell lines with less cell surface expression of LacCer and found that Ba/F3 cell is such a cell line. By repeated sorting for 6 times of high LacCer-expressing cells in Ba/F3, we successfully generated high LacCer-expressing cell, called Lac6. We demonstrated several unbiased screening approaches to reveal molecular mechanisms of GlcCer scrambling.

Results

Using Lac6, CRISPR sgRNA library screening was performed, from which we identified UGCG and β4Galt5, but not scramblases. We then prepared cDNA library using Lac6 cells and expressed in parental cells to identify factors contributing LacCer synthesis. Although some factors were successfully identified, candidate factors for scramblases were not discovered. We then speculated that loss of scramblases in Golgi Apparatus results in growth retardation, causing loss of critical sgRNAs during cell growth. To examine this hypothesis, we performed revival screening using sgRNA library, which enables enrichment of important sgRNAs without growth. Through this screening, finally, we could identify candidate factors for scramblases. In the future, we will investigate whether the identified factors involved in scrambling of GlcCer at Golgi Apparatus.