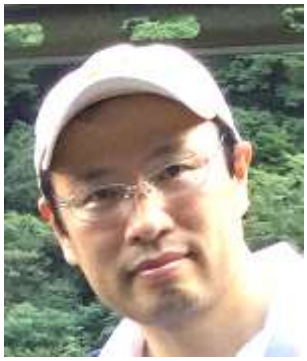
ABSTRACT

Principal Investigator: Toshiyuki Yamaji

Grant Title: Search for new glycosylation-related genes using a CRISPR library and toxins

1. Introduction



Shiga toxin (Stx) and subtilase cytotoxin (SubAB) are virulence factors of Enterohemorrhagic *Escherichia coli* that causes hemorrhagic colitis and hemolytic uremic syndrome. Both toxins utilize glycans for receptors to enter the target cells. Stx recognizes a glycolipid Gb3, whereas SubAB binds sialoglycans on glycoproteins. These toxins cause cell death after retrograde transport to the endoplasmic reticulum. In genetic screens against the cell death induced by these toxins, the identified factors may be categorized into two groups: one is involved in retrograde trafficking, and the other is involved in receptor biosynthesis. In this study, a lentivirus-based CRISPR library was used for genome-wide screening to identify host cell genes that conferred resistance to these toxins when the gene was disrupted.

2. Methods

GeCKO ver2 was used as a lentiviral CRISPR library. The sgRNAs of the CRISPR library were expressed in CAS9-expressed HeLa cells, and the cells were treated with Stx and SubAB. Then, the integrated sgRNA sequences were analyzed with a new-generation sequencer. The genes corresponding to the sgRNAs that were concentrated in the two independent experiments were picked up as the toxin-resistant gene candidates.

3. Results

(1) Stx-resistance screening

About 100 genes were concentrated, and most of the genes were sphingolipid- and membrane trafficking-related. In particular, almost all of the genes directly involved in Gb3 biosynthesis were highly concentrated in this screening, which indicates the high comprehensiveness of this screening. Besides these enzymes, two new genes were identified that conferred strong resistance to Stx when they were disrupted. In the knockout cells of these genes, Gb3 was reduced, and lactosylceramide was accumulated instead, which suggests that both proteins are required for the step of Gb3 synthesis from lactosylceramide.

(2) SubAB-resistance screening

About 60 genes were concentrated, and most were sialoglycan- and membrane trafficking-related genes. Interestingly, not only N-glycan synthesized by MGAT1 but also O-glycans synthesized by C1GalT1 were used as receptors for SubAB (Fig. 1). Although most of the identified membrane trafficking-related genes (e.g. COG complex subunits) were also observed in the Stx screening, several genes showed resistance to SubAB but not to Stx when the genes were disrupted. One of these genes affected sialoglycan biosynthesis.

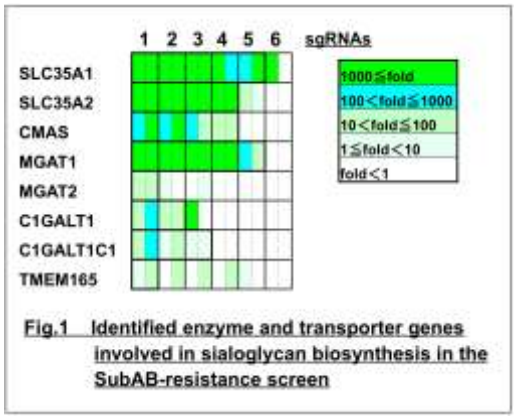


Fig.1 Identified enzyme and transporter genes involved in sialoglycan biosynthesis in the SubAB-resistance screen