Principal Investigator: Ryu Okumura

Grant Title: Elucidation of the mechanism by which FAM3 family protein regulates glycosylation.

Abstract

This study aimed to elucidate how glycan modifications in intestinal epithelial cells contribute to intestinal homeostasis, with a particular focus on the intracellular regulation of glycosyltransferases. Although glycosylation is essential for diverse biological processes, the mechanisms underlying abnormal glycosylation remain incompletely understood. Glycosyltransferases synthesize glycans in the Golgi apparatus; however, how each enzyme maintains its proper Golgi localization has not been fully clarified. Recently, add-on domains of glycosyltransferases have been reported to play important roles in their Golgi localization. However, approximately 80% of glycosyltransferases do not harbor such domains. In this study, we focused on Fam3 family proteins, which show structural similarity to the add-on domain of the glycosyltransferase Pomgnt1. We therefore hypothesized that Fam3 proteins function as auxiliary molecules that regulate the Golgi localization of glycosyltransferases lacking add-on domains.

Among the Fam3 family members, we focused on Fam3b and Fam3d, which are highly expressed in intestinal epithelial cells. In Fam3b-deficient mice, Golgi localization of Fut2 was disrupted, leading to proteasome-dependent degradation of Fut2 and an almost complete loss of α1,2-fucosylation of colonic mucin. Similarly, Fam3d deficiency markedly reduced B3gnt3 protein abundance and impaired the extension of core 1 *O*-glycans. Using FLAG-tag knock-in mice, anti-FLAG immunoprecipitation, proteomics, and western blotting, we found that Fam3b and Fam3d specifically interact with Fut2 and B3gnt3, respectively. Furthermore, the hydrophobic α-helical region at the N terminus of Fam3 proteins was found to function as a Golgi localization signal, revealing a novel mechanism by which Fam3 proteins retain glycosyltransferases in the Golgi apparatus. Both Fam3b- and Fam3d-deficient mice showed markedly increased susceptibility to DSS-induced colitis, demonstrating the importance of these molecules in maintaining intestinal homeostasis. In addition, FAM3B expression was reduced in colonic epithelial cells from patients with ulcerative colitis, suggesting that this mechanism may also be involved in human inflammatory bowel disease.

Together, these findings propose a new concept that abnormal glycosylation can arise not only from altered glycosyltransferase gene expression, but also from impaired auxiliary mechanisms controlling glycosyltransferase localization. This study provides important insight into the spatial regulation of glycan modification and identifies a physiological mechanism that controls Golgi localization of glycosyltransferases at the organismal level. These findings may open a new research field at the interface of glycobiology and mucosal immunology and contribute to future therapeutic strategies targeting glycan modification.