ABSTRACT

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Grant Title: Mechanisms for protein-selective action of GnT-V

Purpose

In this study, focusing on a cancer-related glycosyltransferase GnT-V, we tried to understand the unknown mechanism for its protein-selective action, and to develop GnT-V inhibitors. As a background, we previously solved its crystal structure (*Nat. Commun.*, 2018), and our analysis of several mutants suggests that GnT-V recognizes target proteins through its non-catalytic regions and domains (*BBA Gen. Subj.*, 2020) (*J. Biol. Chem.*, 2022). Based on these findings, we here try to elucidate how GnT-V selects its substrate proteins and design GnT-V-specific inhibitors.

Methods

To accomplish our goals, we focused on 2 points, 1) Mechanism for protein-selective action of GnT-V, 2) Design and synthesis of GnT-V inhibitor. In this project, we collaborate several experts of structural biology (Dr. Masamichi Nagae and Dr. Yoshiki Yamaguchi) and organic chemistry (Dr. Hidenori Tanaka).

Results

1) Mechanism for protein-selective action of GnT-V

Based on the crystal structures, we made models and performed MD simulation to understand how GnT-V recognizes the core part of an acceptor N-glycan. Using several point mutants, we identified a region outside the catalytic pocket plays a key role for recognizing the core part of the acceptor glycan.

Furthermore, using L4-PHA lectin, we biochemically purified the physiological substrates in a mouse organ and identified those proteins by proteomics. We will elucidate the mechanisms by which these substrate proteins are selected by GnT-V in the cells in the future.

2) Design and synthesis of GnT-V inhibitor

Based on the 3D structure of GnT-V, we designed and synthesized new donor substrate analogs for inhibitor candidates. Some of these compounds showed inhibition of GnT-V activity in vitro.