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Inhibition of *N*-Glycosylation towards Novel Anti-Cancer Chemotherapeutics

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Commentary

Cell surface polysaccharides play important roles in numerous biological processes in living organisms such as maintenance of outer membrane integrity, mediators of host-pathogen interactions, cell-cell-adhesion and recognition, protein folding, cell signalling, and trafficking of proteins translated within the rough endoplasmic reticulum (ER) to the Golgi. Furthermore, abnormal glycosylation of cell surface proteins takes place during which normal cells progress to a malignant neoplastic state [1]. Thus, the modification of cell surface glycosylation is a characteristic of many cancer cells [2-5]. Many of the recently developed tumor markers are carbohydrate antigens. Identification of cell type-specific or tissue-specific glycoconjugates (tumor markers) has led to the discovery of new assay systems or diagnosis for certain cancers via immunodetection reagents [1]. On the other hand, anti-glycan antibodies have a limited application for cancer treatment, despite the fact that a great number of tumor-associated glycans have been identified with the help of modern glycomic approaches [6]. In the last two decades, a few monoclonal antibodies targeting ganglioside GD_2 or GD_3 and a cancer vaccine with *N*-glycolylated ganglioside GM_3 have been developed into clinical trials. As such, changes and diversification of the expression profile of cell surface glycans based on the underlying glycobiology have received much attention from the scientific community [1]. Two of the most abundant forms of glycosylation occurring on proteins destined to be secreted or membrane-bound proteins are *N*-linked (to Asp (N), *N*-glycosylation) and mucin-type *O*-linked (to Ser/Thr, *O*-glycosylation). *O*-Linked glycosylation is an evolutionarily conserved protein modification found across species such as mammals, worms, insects, protozoa, and certain types of fungi, whereas *N*-linked glycosylation occurs in eukaryotes and widely in archaea, but very rarely in bacteria. Recent studies of cancer immunotherapy are based on immunogenicity of truncated *O*-glycan chains (e.g. Tn, sTn, T, and sLea/x). Despite the importance of *N*-linked glycans in transformation-associated glycosylation changes for normal cells to develop tumor cells, therapeutic antibodies against *N*-linked glycans have not yet been developed. It may largely be attributable to the lack of specificity of *N*-linked glycans between normal and malignant cells. Abnormal (increased) branching of *N*-linked glycans has been observed in certain solid cancer cells. Altered glycosylation of *N*-linked glycans in cancers is typically associated with upregulation of β1,6-*N*-acetylyglucosaminyltransferase-5 (GnT5), enhancing β1,6-branching. Although it is an extremely challenging subject to discover drug-like glycosyltransferases to block the biosynthesis of specific branching processes in cancer cells, *N*-glycan biosynthesis can be terminated by inhibition of the first committed enzyme, dolichyl-phosphate *N*-acetylglucosaminephosphotransferase (DPAGT1) activity [7-11]. Selective DPAGT1 inhibitors have the promising therapeutic potential for certain solid cancers that require increased branching of *N*-linked glycans in their growth progressions. This editor's note summarizes overall perspective of DPAGT1 inhibitors as anticancer chemotherapy.

DPAGT1, which belongs to the glycosyltransferase family 4, is an integral membrane protein localized in the ER that catalyzes the transformation from UDP-GlcNAc to *N*-acetyl-D-glucosaminyl-diphosphodolichol with dolichyl phosphate. Anchored *N*-acetyl-D-glucosaminyl-diphosphodolichol in the ER membrane is modified by sequential glycosyltransferases to form dolichol-linked oligosaccharide precursors that are transferred to selected asparagine residues (*N*-X-S or N-X-T sequences) of polypeptide chains by oligosaccharyltransferase (OST).

β-Catenin, encoded by the *CTNNB1* gene (a proto-oncogene), is a multifunctional that regulates and coordinates cell-cell adhesion and gene transcription. β-Catenin is a crucial transcriptional factor in highly conserved Wnt (Wingless-Int)/β-catenin signaling pathway, and plays an important role in embryonic development and carcinogenesis [3-12]. In normal cells, β-catenin concentration is low because of proteasome degradation. The mutations of β-catenin are found in a variety of cancers including ovarian cancer, breast cancer, cancerous liver tumors, colorectal cancer, lung cancer and glioblastoma [12]. In these cancer cells, the mutations are observed in the β-transducin repeat-containing protein (β-TrCP) binding motif that facilitates ubiquitinylation, making degradation of β-catenin difficult. It causes a high level of β-catenin in the cytoplasm, which is translocated to the nucleus and drives transcription of the target genes including *Wnt* genes. An alternative function of β-catenin and the other member of the catenin protein family (α-catenin, and β-catenin (plakoglobin)) are linked to E-cadherin, a calcium-dependent cell-cell adhesion molecule that responsible for intercellular cell-adhesions. One of the *N*-glycosylation targets of DPAGT1 is E-cadherin. Overexpression of β-catenin causes a high level of *DPAGT1* expression, leading to abnormal modification of E-cadherin. Numerous studies concluded that the Wnt/β-catenin signaling pathway regulates the metabolic pathway of protein *N*-glycosylation by targeting *DPAGT1* expression. Dysregulation of DPAGT1 causes disturbances in intercellular adhesion in oral cancer [13]. Based on these observed biological processes (Wnt/β-catenin pathway, high level of *DPAGT1* expression followed by abnormal *N*-linked glycaosylation of E-cadherin in growth of certain cancer cells), inhibition of DPAGT1 may induce the loss of cell-cell adhesion and metathesis, and trigger an apoptotic pathway [14]. Due to the fact that only a few DPAGT1 inhibitor molecules have been identified to date, in vitro studies associated with *N*-linked glycosylations through DPAGT1 and protein misfolding have been performed with an antibiotic, tunicamycin. Tunicamycin belongs to nucleoside antibiotics that inhibit phospho-MurNAc-pentapeptide translocase (translocase I or MraY) and polyprenyl phosphate-

GlcNAc-1-phosphate transferase (WecA, an orthologue of DPAGT1) enzymes. These enzymes are responsible for biosynthesis of peptidoglycan and cell wall (e.g. lipopolysaccharide in Gram-negative and arabinogalactan-mycolic acid in *Mycobacterium spp.*), respectively [15]. It is interesting to note that tunicamycin shows >10 times stronger inhibitory activity against WecA (IC_{50} 0.120 ± 7.80 μg/mL) than that of MraY (IC_{50} 2.73 ± 0.138 μg/mL). Unfortunately, eukaryal phosphotransferase (e.g. Alg7 and DPAGT1) inhibitory activities of tunicamycin are rather moderate (IC_{50} 10~25 μM) [16]. An alternative mechanism of cytotoxicity of tunicamycin is due to its perturbation ability of cell membrane structure. However, several studies regarding plasma membrane transporters suggest that tunicamycin requires uptake through a membrane transporter (i.e. MFSD2A), and thus, non-specific toxicity caused by the cell membrane perturbation may be reduced by a specialized transporter mechanism when applied at lower concentrations (<<10 μM) than the non-specific toxicity levels. Tunicamycin is not a therapeutic agent that is tolerated for *in vivo* studies. Tunicamycin displays a wide range of biological activities associated with ER stress, protein misfolding and ATP-binding cassette subfamily G member 2 (ABCG2). Thus paraptosis/apoptosis inductions observed by addition of tunicamycin may not be caused solely by the inhibition of DPAGT1. As of today, over 100 scientific articles have been discussed about anticancer effects of tunicamycin. Among the widespread application of tunicamycin in the laboratories, quite a few studies have addressed anticancer activity of tunicamycin by targeting the *N*-linked glycan biosynthesis. At 0.3~1.2 μM concentrations, tunicamycin can suppress migration and invasion of a human colon cancer cell line, HCT116 by an anchorage-dependent colony formation [17]. Their studies may demonstrate that inhibition of *N*-linked glycosylation by tunicamycin prevents the migration and adhesion of the colon cancer cells.

In summary, although a number of tumor-associated *N*-glycan has been identified, development of therapeutic antibodies against *N*-linked glycans remains a very challenging subject. Specific *N*-linked glycan biosynthesis inhibitors have the potential to be new drugs for certain cancers that requires increased branching of *N*-linked glycans for cell growth and metastasis. DPAGT1 catalyzes the first step in the dolichol-linked oligosaccharide pathway, leading to the syntheses of complex *N*-linked glycan. Unlike other cytosolic glycosyltransferases, DPAGT1 has the large hydrophobic catalytic domains which have beneficial for discovering drug-like small molecules. Tunicamysin is a strong bacterial WecA inhibitor that shows a narrow-spectrum of antibacterial activity (against some Gram-positive bacteria including *M. tuberculosis*). A sufficient amount of DPAGT1 enzyme purification for compound screening is not feasible due to lack of chemical stability. On the other hand, a high-throughput screening (HTS) against WecA enzyme has been established recently [15]. Chemical synthesis of a library of complex nucleoside analogs is practical via modern synthetic method. Thus, a sequential assay screening of WecA inhibitors via HTS followed by cross-reactivity testing with DPAGT1 will identify new inhibitor molecules that inhibit *N*-linked glycan biosynthesis. New DPAGT1 inhibitors identified from synthetic library molecules should be optimized through exhaustive medicinal chemistry effort to fulfil *in vitro* activity, toxicity, basic pharmacokinetic property for *in vivo* studies. Inhibition of DPAGT1 may very well be the "Achilles' heel" of the biosynthesis of essential *N*-glycan in certain cancers.

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