C1GALT1 in health and disease (Review)

XIAOJIE SUN[1], MENGRU ZHAN[2], XUN SUN[3], WANQI LIU[1] and XIANGWEI MENG[1]

Departments of [1]Gastroenterology, [2]Hepatobiliary and Pancreatic Medicine, and [3]Pathology, The First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China

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Abstract. O-linked glycosylation (*O*-glycosylation) and N-linked glycosylation (*N*-glycosylation) are the two most important forms of protein glycosylation, which is an important post-translational modification. The regulation of protein function involves numerous mechanisms, among which protein glycosylation is one of the most important. Core 1 synthase glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1 (*C1GALT1*) serves an important role in the regulation of *O*-glycosylation and is an essential enzyme for synthesizing the core 1 structure of mucin-type O-glycans. Furthermore, *C1GALT1* serves a vital role in a number of biological functions, such as angiogenesis, platelet production and kidney development. Impaired *C1GALT1* expression activity has been associated with different types of human diseases, including inflammatory or immune-mediated diseases, and cancer. *O*-glycosylation exists in normal tissues, as well as in tumor tissues. Previous studies have revealed that changes in the level of glycosyltransferase in different types of cancer may be used as potential therapeutic targets. Currently, numerous studies have reported the dual role of *C1GALT1* in tumors (carcinogenesis and cancer suppression). The present review reports the role of *C1GALT1* in normal development and human diseases. Since the mechanism and regulation of *C1GALT1* and *O*-glycosylation remain elusive, further studies are required to elucidate their effects on development and disease.

Correspondence to: Professor Xiangwei Meng, Department of Gastroenterology, The First Hospital of Jilin University, 71 Xinmin Street, Changchun, Jilin 130021, P.R. China
E-mail: mengxw@jlu.edu.cn

Abbreviations: *O*-glycosylation, O-linked glycosylation; *N*-glycosylation, N-linked glycosylation; ER, endoplasmic reticulum; GalNAc, N-acetylgalactosamine; C1GALT1, core 1 synthase glycoprotein-GalNAc 3-β-galactosyltransferase 1; O-GalNAc, GalNAc type *O*-glycosylation; Tn antigen, Thomsen-nouveau antigen (GalNAc-α-1-R); T antigen, Thomsen-Friedenreich antigen (Gal-β-1-3GalNAc-R); ESCC, esophagus squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma; EGFR, epidermal growth factor receptor; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; FAK, focal adhesion kinase; IgAN, IgA nephropathy; Gd-IgA1, galactose-deficient immunoglobulin A1; PDAC, pancreatic ductal adenocarcinoma

Key words: C1GALT1, *O*-glycosylation, integrin β1, Cosmc, itraconazole

Contents

1. Introduction

Dynamic regulatory mechanisms under a variety of physiological conditions affect the processing and maturation of proteins in mammalian cells. Glycosylation is an important type of post-translational modification. More than half of the proteins in human cells and 50-70% of serum proteins are glycosylated proteins (1). Mucin-type O-linked glycosylation (*O*-glycosylation) and N-linked glycosylation (*N*-glycosylation) are the two most important forms of glycosylation, and changes in either can lead to clinically significant pathogenesis (2-4). *O*-glycosylation is considered a protein modification occurring on proteins that are secreted and membrane-bound; it serves a key role in protein processing, secretion, stability, and ligand binding (5). *O*-glycosylation is associated with different types of biological processes, such as metabolism, translation, transcription, cytoskeletal formation, cell cycle progression and cell signal transduction (6,7). Abnormal *O*-glycosylation is associated with a number of human diseases, including the development of tumors (8). Tumor cells often contain numerous altered *O*-glycosylated proteins, which qualitatively and/or quantitatively change sugar molecule expression (9). Some *O*-glycosylated proteins are usually adopted as tumor biomarkers in the circulation, such as cancer antigen (CA) 19-9 and CA-125 (9).

O-glycosylation of proteins most commonly occurs in the serine and threonine residues, but it can also occur in the tyrosine, hydroxylysine and hydroxyproline residues. Glycosylation is initiated in the endoplasmic reticulum (ER) (10). However,

O-Xyl (proteoglycan) and N-acetylgalactosamine (GalNAc) type *O*-glycosylation (O-GalNAc) are initiated in the Golgi apparatus (10). More than 80% of cell membrane proteins and extracellular secreted proteins are O-GalNAc glycosylated proteins (11). This type of glycosylation is mediated through transferring GalNAc from UDPGalNAc to threonine or serine residues. The GalNAc aminotransferase peptide, which contains up to 20 isoenzymes, catalyzes this reaction (10,12). The protein encoded by core 1 synthase glycoprotein-GalNAc 3-β-galactosyltransferase 1 (*C1GALT1*) generates the common core 1 O-glycan structure, Gal-β-1-3GalNAc-R (T antigen), by the transfer of galactose (Gal) from UDP-Gal to GalNAc-α-1-R (Tn antigen) (13). The formation of complex O-glycan structures requires further modification of the T antigen (14). To date, at least eight different O-glycan core structures have been described. The core 1 type O-glycan forms the basis of *O*-glycosylation modification and its synthesis is mainly regulated by C1GALT1 (15). The Tn antigen can form core 3 structure under the catalysis of β1,3-N-acetylglucosaminyltransferase 6. Core 1 and 3 structures are catalyzed by β1,6-N-acetylglucosa minyltransferase to form core 2 and 4, respectively (16).

There are three species of β1,6-N-acetylglucosaminyltransferases in mammals: Two of them catalyze the core 2 structure constitution, and one catalyzes the constitution of core 2 or 4 structures (17) (Fig. 1). In addition, there are several other core structures that are less common than the aforementioned ones (17).

The *C1GALT1* gene is located on chromosome 7p22.1-p21.3, and the protein T-synthase, encoded by the gene, has been considered to be a core mucin-type *O*-glycosyltransferase that resides in the Golgi apparatus (17), and is synthesized due to its particular chaperone, Cosmc, in the ER (18). Aryal *et al* (18) reported that T-synthase could co-immunoprecipitate with Cosmc. Cosmc can interact with the deactivated T-synthase, partially restoring the enzyme activity *in vitro*, and is a specific partner of T-synthase folding and maturation (18). This enzyme adds β1,3-bonded galactose to the existing GalNAc to produce a common core O-glycan structure. Core 1 is the precursor of many cell surface mucin O-glycans and secreted glycoproteins, and is the basis for the formation of complex O-glycans, such as core 2 structure and sialylated T antigens (19). Furthermore, *C1GALT1* serves a vital role in numerous biological functions, including angiogenesis, platelet production and kidney development (20,21).

The expression of normal O-glycans is associated with health and homeostasis, whereas abnormal glycosylation is associated with cancer and other pathologies. Abnormal glycosylation is involved in cancer cell invasion, migration, angiogenesis, intercellular contact and epithelial-mesenchymal transition (EMT) (1,22-24). Previous studies have revealed that changes in the level of glycosyltransferase are associated with cancer (25,26). In addition, *C1GALT1* has been associated with the metastasis and progression of various types of cancer, such as liver and gastric cancer (27,28).

C1GALT1 can act as an oncogene or a tumor suppressor gene in various types of conditions. C1GALT1 high expression in liver cancer tissues is associated with advanced tumors, poor prognosis and metastasis (29). On the contrary, another study has presented C1GALT1 as a tumor suppressor gene in various types of tumors (30).

Chugh *et al* (30) discovered that *C1GALT1* expression is higher in well-differentiated pancreatic cancer tissues compared with in poorly differentiated pancreatic cancer tissues. Moreover, in cancer tissues, T antigen expression is lower compared with that of the Tn antigen (30). *C1GALT1* has been shown to be a tumor suppressor gene in pancreatic cancer since the absence of *C1GALT1* expression promotes the development and metastasis of pancreatic cancer (30). However, in another study, *C1GALT1* served a different role (31). Liu *et al* (31) found that in *C1GALT1*-knockout mice, spontaneous gastroenteritis and consequently gastric antral adenocarcinoma were improved in the gastric mucosal epithelial cells, which indicates that *C1GALT1*-mediated *O*-glycosylation is very important for gastric mucosal and gastric homeostasis protection (31). However, the use of samples from different sources or at different tumor stages may have contributed to the observed dual function of *C1GALT1*; hence, the true role of the gene remains elusive. The present review focuses on the role of *C1GALT1* in health and disease.

2. *C1GALT1* in normal development and non-neoplastic diseases

C1GALT1 in normal development. C1GALT1 and glycosylation are essential for normal development, especially during angiogenesis, platelet production and kidney development (32). Impaired T-synthase activity has been associated with different types of human diseases, including inflammatory or immune-mediated diseases, and cancer (33).

Xia *et al* (20) targeted deletion of the *C1GALT1* gene, resulting in normal development of heterozygous mice and the mating of 364 viable offspring. A total of 228 (63%) *T-syn*+/- progenies and 136 (37%) *T-syn*+/+ progenies were identified by genotyping, but no *T-syn*-/- progenies were identified (20). Whether deletion of two alleles of C1GALT1 led to fetal death of 293 (E9-16) embryos was analyzed (20). Genotyping revealed the offspring of 142 (48%) *T-syn*+/-, 78 (27%) *T-syn*+/+ and 73 (25%) *T-syn*-/- (20). T-synthase activity was decreased in *T-syn*+/- embryos at E12, and there was no activity in *T-syn*-/- embryos (20). These results confirm that all active T-synthase are encoded by *C1GALT1* at this stage of development. T-synthase was found to be different from the typical glycosyltransferase (34). *T-syn*-/- embryos in E9 developed normally, but then they gradually developed significant bleeding in the spinal cord and brain. The *T-syn*-/- embryos all died at E13 or E14 (20). In the *T-syn*-/- embryos, the only exception detected was poor angiogenesis (19,20). This phenomenon may be explained by isolation of endothelial cells from extracellular matrix and supporting pericytes (20). If mice lack growth factor B, they cannot recruit peripheral cells to the developing cerebral vessels, and bleeding will occur in late embryo or perinatal period (35). By contrast, the *T-syn*-/- embryo always died at E14; this means that in the process of angiogenesis, one or more endothelial proteins are inseparable from core-1-derived O-glycans (20). This possibility may be further explored by constructing an endothelial cell model of *C1GALT1*-targeted deletion (21).

Although O-glycans are considered to be ubiquitous in various tissues and types of cells, the expression of hematopoietic and endothelial cells is high throughout postpartum and

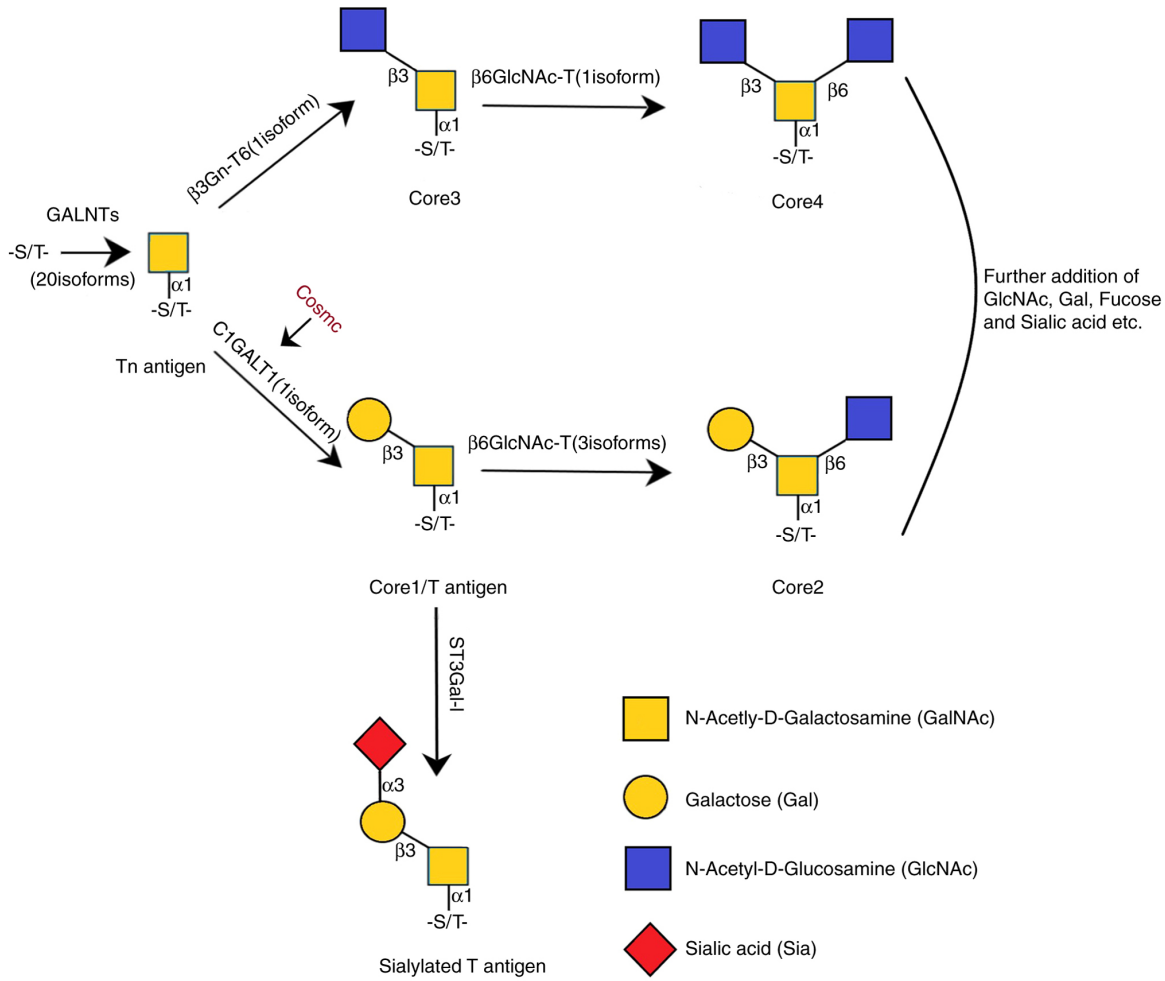

Figure 1. *O*-glycosylation model. C1GALT1 transfers Gal from UDP-Gal to Tn antigen to form core 1 O-glycan structure, T antigen. Core 1 is a precursor for numerous extended mucin-type O-glycans on the cell surface and secreted glycoproteins. The structure of core 3 is catalyzed by β1,3-N-acetylglucosaminyltransferase 6. Core 1 and 3 core structures can be further modified to form the structure of core 2 and 4, respectively, by the catalysis of β1,6-N-acetylglucosaminyltransferases. Gal, galactose; GalNAc, N-acetylgalactosamine; C1GALT1, core 1 synthase glycoprotein-GalNAc 3-β-galactosyltransferase 1; GlcNAc, N-acetylglucosamine; GALNT, N-acetylgalactosaminyltransferase.

embryonic development (15,36,37). Fu *et al* (21) generated mice lacking T-synthase specifically in endothelial and hematopoietic cells (named EHC-T-syn-/- mice model). The Tn antigen can be expressed in hematopoietic, lymphatic and endothelial cells and arteries, but not in other types of cells (21). The mice developed lymphatic vessel defects and abnormal lymphatic function. Unlike mice with complete *C1GALT1*-knockout, EHC-T-syn-/- mice have no 'cerebral hemorrhage' and 'partial onset' embryonic lethality (21). The phenotypic difference may be due to O-glycans of other types of cells, such as nerve cells and parietal cells, which contribute to blood vessel development in nerve tissues (21). However, EHC-T-syn-/- mice exhibited high neonatal mortality, vascular system disorder and impaired lymphatic function (21). At the time of autopsy, ~75% of EHC-T-syn-/- mice exhibited extensive small intestinal bleeding, which may be one of the reasons for the lethality of EHC-T-syn-/- after birth (21). Abnormal blood vessels in the blind end of EHC-T-syn-/- mice exhibited abnormal function/development of lymphatic vessels, constituting abnormal links between lymphatic vessels and blood (21). These hyperemic lymphatic vessels are found in mice that lack fasting-induced adipokines and have defects in the signaling proteins SLP-76 and SYK (38). These observations suggest that constant O-glycoprotein expression is required for the maintenance of lymphatic vessels, angiogenesis and the separation of blood and lymphatic vessels during development.

In addition, *C1GALT1* is very important in the formation of the follicular basal layer (FBL) and the follicular environment. The basement membrane provides structural and selective filters for molecules. The environment is regulated by the FBL in the follicle at the time of development (39). It has been demonstrated that the oocyte is important in producing FBL (40). Mice with *C1GALT1* oocyte-specific deletion do not synthesize main 1β1,3-galactosyltransferase 1 (named T-synthase as well), and thus are not able to constitute main 1 derived O-glycan (41). Therefore, the FBL changes the distribution of laminin and collagen (39) and causes the follicles to combine to form multiple follicles, with two or more oocytes in a follicle. Therefore, *C1GALT1* expression serves a part in keeping the normal structure of FBL and the follicular environment.

Additionally, a series of experiments have revealed that *C1GALT1* is very important for platelet production and renal homeostasis. Kudo *et al* (42) conditionally knocked out

C1GALT1 and constructed 'Mx1-C1' mice, so that the deletion of the *C1GALT1* gene was limited to bone marrow cells. Mx1-C1 mice exhibited severe thrombocytopenia. The hematology parameters indicated a marked decrease in platelet count. However, white and red blood cell counts, as well as the levels of hemoglobin, were normal. Notably, giant platelets were in the peripheral blood smear, while the morphology of other cells was normal. Compared with the platelets of wild-type (WT) mice, those of Mx1C1 mice were larger. Tail bleeding time measurement indicated that bleeding in WT mice was prevented 6 min after cutting the tail, while bleeding time in Mx1-C1 mice was markedly prolonged (>10 min), indicating that *C1GALT1* expression is important for hemostasis and platelet production (42).

In plt1 mice constructed by Alexander *et al* (32), T-synthase showed residual enzyme activity, and through a series of experiments, it was revealed that *C1GALT1* had a very important role in platelet production and renal homeostasis. Alexander *et al* (32) treated C57BL/6 mice with N-Ethyl-n nitrosourea (ENU) and produced generation III (G3). In lineage 76, multiple mice exhibited lower platelet counts, consistent with the isolation of ENU-induced mutations that cause thrombocytopenia. Lineage 76 with recessive mutation was called plt1 (32). plt1/plt1 mice had 40% of the platelet count of WT mice, and all major organs were histologically normal except the kidneys, which exhibited structural distortion of the glomerulus-renal tubules (32). The levels of creatine, blood urea and urinary protein in plt1/plt1 mice were higher compared with those in WT mice. The kidneys exhibited inflammatory infiltration, ductal stenosis, glomerular loss and cortical atrophy. From the 10th week, plt1/plt1 mice began to get sick, and by day 200, 90% of the mice had died (32). It was demonstrated that the activity of T-synthase in plt1/plt1 mice was <5% of that in WT mice. Plt1 mutation could lead to severe but incomplete loss of T-synthase activity (32).

Decrease of T-synthase activity can lead to exposure to the Tn antigen. The Tn antigen could not be detected in WT mice, but could be detected in plt1 mice. plt1/plt1 mice died of a severe kidney disease accompanied by massive proteinuria and glomerulonephritis (32). Podocalyxin, which develops from podocytes of the kidney, has been discovered to be a core TN protein in the kidney (43). Mice with low levels of podocalyxin shortly died after birth from anuria and renal dysplasia, consistent with the anti-adhesion effects of podocalyxin on the podocyte surface for ensuring that the filtration gap is obstructed (44). Plt1/plt1 mice can produce urine, which proves that low glycosylated podocalyxin can maintain part of renal function. However, kidney disease in mice indicates that podocalyxin glycosylation mediated by T-synthase is crucial for the maintenance of normal renal function and structure (32). These results suggest that some pathological changes in the kidney may be associated with a decrease of T-synthase activity, which does not depend on the influence of intrinsic defects and immune factors. In addition to kidney diseases, further attention should be given to IgA nephropathy (IgAN).

IgAN. The decreased activity of T-synthase has a close association with human diseases, the most notable being IgAN (an ordinary important glomerulonephritis). IgAN has been considered to be the most common reason of renal failure and glomerulonephritis globally, and it is an immune-mediated disease characterized by abnormal glycosylation (45). IgAN accounts for 37-58% of biopsy-confirmed primary glomerulonephritis in China (46-48). Within 10 years after diagnosis, approximately one-third of patients with IgAN will progress to the final stage of kidney disease (49,50). Two case-control studies have discovered that Chinese population susceptibility and *C1GALT1* gene polymorphism are associated with the IgAN variations of the *C1GALT1* gene; in particular, the haplotypes YATIG, YAGDA and YATDG were associated with the susceptibility to IgAN (51,52). Abnormal *O*-glycosylation of IgA1 has been identified in IgAN, which was an important breakthrough in the study of its pathogenesis (53). IgA1 glycosylation defects result in elevated galactose-deficient IgA1 (Gd-IgA1) and immunocomplex, and are associated with IgAN development (53).

There is evidence that the Gd-IgA1 level is heritable (54,55). Using a genome-wide approach, Gale *et al* (56) identified common genetic factors that influence Gd-IgA1 levels in East Asian and Caucasian populations. Gale *et al* (56) studied hundreds of patients with IgAN from the UK and China, revealing that *C1GALT1* is an important genetic determinant of Gd-IgA1 level, which is an independent risk factor for progressive IgAN. Compared with that in ethnicity-matched healthy subjects, the Gd-IgA1 level is increased in patients with IgAN and is associated with disease severity (56). Chinese patients with IgAN have lower levels of Gd-IgA1 than Caucasian patients (56). This suggests that there may be ethnical differences in the pathogenic importance of IgA1 *O*-glycosylation changes.

Kiryluk *et al* (57) used *in vitro* small interfering (si)RNA knockdown to demonstrate that *C1GALT1* can determine the secretion rate of Gd-IgA1 in serum IgA1-producing cells. Xing *et al* (58) discovered that *C1GALT1* expression in peripheral B lymphocytes of patients with IgAN has a negative correlation with increased Gd-IgA1 levels and is markedly downregulated compared with the increase of Gd-IgA1 level. The aforementioned study involved 30 patients with IgAN and 30 healthy volunteers in China (58). Gd-IgA1 level was measured by an enzyme-linked immunosorbent assay, and the results revealed that Gd-IgA1 levels ranged between 8.55 and 14.48 U/ml in patients with IgAN and between 3.97 and 12.15 U/ml in healthy controls (58). In comparison with those in healthy controls, Gd-IGA1 levels were determined to be significantly higher in patients with IgAN ($P<0.001$) (58). By reverse transcription-quantitative PCR, the expression levels of *C1GALT1* were detected in peripheral B lymphocytes of both patients with IgAN and healthy controls, revealing that *C1GALT1* expression was significantly downregulated in patients with IgAN compared with that in healthy controls ($P=0.04$) (58). It has been suggested that a decrease in *C1GALT1* expression in B lymphocytes may contribute to the increased production of Gd-IgA1 and eventually lead to IgAN pathogenesis (59). One difficulty in exploring the role of *C1GALT1* in IgAN is that only a small proportion of plasma cells secrete IgA1, which is associated with the disease. The identification and isolation of these plasma cells are difficult, but it is important for elucidating the role of *C1GALT1* in IgAN. Studying the real cause of the lack of *C1GALT1* expression may illustrate the pathogenesis of IgAN and contribute to finding new treatments for the disease.

Tn syndrome. In addition to IgAN, another disease that is closely associated with decreased T-synthase activity is Tn syndrome. Tn syndrome is an infrequent blood disorder characterized by exposure of the Tn antigen on the surface of human red blood cells, granulocytes, platelets and lymphocytes (60). Patients can present as asymptomatic, or can exhibit mild hemolysis, thrombocytopenia and/or leukopenia, which are usually considered to be caused by the reaction of Tn antigens with naturally occurring anti-Tn antibodies (61). These antibodies may be IgM condensation agglutinin-type and appear to be autoantibodies against carbohydrate I antigens on adult red blood cells (62). Another possible pathological mechanism is the abnormal function of glycoproteins on leukocytes or platelets. Since glycoproteins have an important role in the function of these cells, changes in glycosylation may impair the function of glycoproteins (62).

The expression of Tn antigen and T-synthase activity loss is the result of Cosmc mutation, which has been widely confirmed (36,63-66). A study by Vainchenker *et al* (60) has demonstrated the existence of the Tn antigen on stem cells of the Tn clone, and Tn syndrome is derived from acquired somatic changes in Cosmc in the early blood progenitor cells. Wang *et al* (67) constructed a mouse model with a targeted deletion of Cosmc in hematopoietic cells/endothelial cells (EHC Cosmc$^{-/y}$), which caused fatal perinatal bleeding in ~90% of mice. The surviving mice developed macrothrombocytopenia and severely prolonged caudal bleeding time (67). Compared with those in wild-type (Cosmc$^{+/y}$) mice, platelets in EHC Cosmc$^{-/y}$ mice were lacking T-synthase activity. The decrease in T-synthase activity was associated with the expression of the Tn antigens on the surfaces of most platelets from EHC Cosmc$^{-/y}$ mice (67). These experiments convincingly suggest that thrombocytopenia and hemorrhage in patients with Tn syndrome are primarily caused by the lack of functional Cosmc.

High expression of Tn antigen is associated with Tn syndrome, as well as with cancer (68). According to statistics, >70% of human cancers may express Tn antigen, including colon (69), breast, ovarian and uterine cervical epithelial cancer (70-72). The expression of Tn antigen is closely associated with a poor prognosis, and it is an attractive target for the development of new diagnostic and therapeutic methods (70).

Inflammatory bowel disease. Inflammatory bowel disease (IBD), consisting of ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disease. Although the exact cause of IBD remains unclear, it is generally believed to be jointly caused by environmental factors and genetic susceptibility. At the same time, intestinal microorganisms serve an important role in the occurrence and development of IBD (73).

The colonic mucus layer is divided into two layers. The inner layer adheres to epidermic cells, and in healthy conditions it is impermeable to bacteria. The primary mucin (MUC) secreted by colon cells is MUC2, which is generally *O*-glycosylated (74) (Fig. 2). Active human UC is associated with a mucus layer with structural and functional defects, such as a thinner mucus layer and increased permeability to bacteria (75,76). Studies have reported that patients with active UC have lower levels of carbohydrates in their mucus layer compared with those in healthy controls and patients with

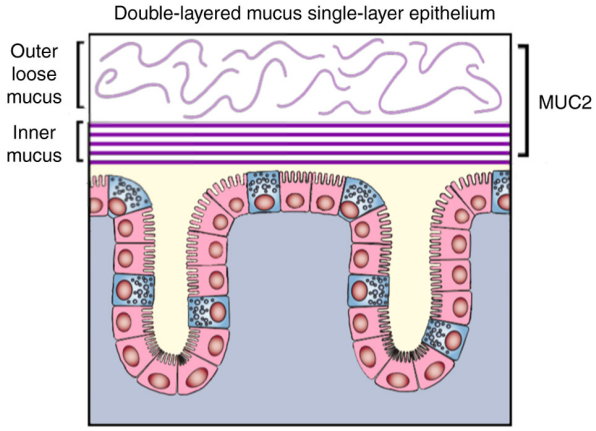

Figure 2. Two-layer mucus system in the colon with a loose, unattached outer mucus layer and a tightly attached inner layer. MUC2, mucin 2.

dormant disease (77-79). Defects in the inner mucus layer can result in increased bacterial association with epithelial cells, which may trigger inflammation (80). In serum, reduced galactosylation of IgG is considered a diagnostic marker for IBD disease (80). The function of suitable mucin glycosylation is also proven by the fact that mice defective in core 1derived Oglycans have poor glycosylated MUC2 and develop spontaneous colitis resembling UC (75).

Fu *et al* (75) established a mouse model of colitis evoked by intestinal epithelial cells lacking *C1GALT1*. The clinical manifestations and pathological features are very similar to those observed in humans (75). The mice developed transient colitis immediately at 3 weeks of age, which subsided at 6 weeks, but relapsed at 8 weeks; the severity of the disease could be reduced by broad-spectrum antibiotic treatment in mice with metronidazole and vancomycin (75). Additionally, the mice exhibited colon tumors when they were older. Immunohistochemistry and histology proved that these tumors were invasive adenocarcinoma, and the tumor tissue expressed abundant Tn antigen (75). The association between genetic variations in *C1GALT1* and the microbiota in hundreds of patients with CD and healthy controls has also been studied (81). Polymorphisms around *C1GALT1* (rs10486157) and *COMSC* (rs4825729) have been associated with changes in the composition of the microbiota of the colonic mucosa (81). These results support the association between *C1GALT1* or *O*-glycosylation and host regulation of the microbiome and suggest a role for the intestinal microbiome in the pathogenesis of IBD. Improvements in understanding the molecular etiology of IBD, especially pathways involving glycans, may facilitate the development of therapeutic drugs.

The high embryonic lethality exhibited by *C1GALT1*-knockout mice prevents the development of an effective *C1GALT1* deficiency animal model. Simultaneously, it also demonstrates that *C1GALT1* and *O*-glycosylation are vital in normal development. One study has demonstrated that numerous membrane glycoproteins expressing Tn antigen and/or truncated O-glycans may be dysfunctional due to degradation and/or folding errors (82). Therefore, the expression of normal O-glycans is associated with health and homeostasis, while the truncation of O-glycans and Tn antigens is associated with pathologies. The association between the role of

C1GALT1 in angiogenesis, platelet production and kidneys, and the pathways it may regulate requires further research.

3. *C1GALT1* as an oncogene

C1GALT1 functions as an oncogene in some cases. The role of the gene in tumor cells and its association with different types of related signaling pathways and molecules have been shown in previous research.

Liver cancer. It is known that *O*-glycosylation can regulate receptor tyrosine kinases (RTKs), such as fibroblast growth factor receptor 2, MET and epidermal growth factor receptor (EGFR) (27,83-86). Changes in RTK activities are associated with cancer progression and occurrence (27). The hepatocyte growth factor (HGF)/c-Met signaling pathway is important in tumor invasion and metastasis (87). The HGF/c-Met axis is involved in cell proliferation, movement, differentiation, invasion, angiogenesis and apoptosis via activation of multiple downstream signaling pathways (87-89). *C1GALT1* can activate the HGF/c-Met signaling pathway and increase mucin O-glycan expression in liver cancer cells, which promotes the proliferation of cells (27). High protein and mRNA expression levels of *C1GALT1* are usually associated with a poor prognosis and metastasis in hepatocellular carcinoma tumors (27). Overexpression of *C1GALT1* in hepatocellular carcinoma activates the HGF signaling pathway through the regulation of dimerization and *O*-glycosylation level of the MET protein (27). Additionally, *C1GALT1* expression can regulate the proliferation and viability of hepatoma cells both *in vivo* and *in vitro* (27).

Wu *et al* (27) reported that C1GALT1 enhanced cell proliferation triggered by HGF through MET. The aforementioned study revealed that *C1GALT1* expression was upregulated in hepatocellular carcinoma. According to the immunohistochemical analysis of 32 non-tumor liver tissues and 72 primary hepatocellular carcinoma tissue specimens, *C1GALT1* expression was upregulated in 54% of hepatocellular carcinoma tissues, but only in 19% of non-neoplastic liver tissues (Mann-Whitney U test, P=0.002) (27). Compared with non-tumor liver tissues, *C1GALT1* expression was frequently upregulated in hepatocellular carcinoma tumors. High *C1GALT1* expression was associated with poor prognosis and tumor metastasis (27). Moreover, the study revealed an important correlation between the expression levels of phospho-MET and C1GALT1 (R=20.73, P<0.0001) (27). Additionally, MET dimerization and phosphorylation were decreased by knocking out C1GALT1 in hepatocellular carcinoma cells, and MET HGF-induced activation was enhanced by C1GALT1 overexpression (27). The trypan blue rejection test revealed that C1GALT1-enhanced cell viability was significantly inhibited by blocked MET activity (27). The proliferation of the cells was decreased by knocking out C1GALT1 through HGF (27). On the contrary, the HGF-induced cell proliferation was enhanced by C1GALT1 overexpression (27). Therefore, the aforementioned study offers new insights into glycosylation in the regulation of RTK activities.

Gastric cancer. According to preclinical patterns of gastric cancer, activation of the HGF/c-Met signaling pathway is able to improve EMT (27,90); nevertheless, further studies are required to determine whether *C1GALT1* can promote tumor malignancy or activate the HGF/c-Met signaling pathway in gastric cancer cells. One study has revealed that changes in RTK genome have been observed in ~37% of patients with gastric cancer (91). The occurrence and development of gastric cancer is promoted actively by RTK, which is considered as a target for cancer treatment (92,93).

The ephrin (EPH) receptor is the largest of the RTK family and is usually upregulating in tumors, which promotes tumor development (94-97). These receptors are popular drug targets (98,99). The human EPH receptor consists of a neighboring EPHA and five EPHB domains. Ephrin A1 is a ligand of the EPHA receptor and has been shown to be upregulated in gastric cancer, promoting EMT (100,101). Lee *et al* (28) observed that *C1GALT1* expression increased in gastric adenocarcinoma and was associated with a poor prognosis. Soluble ephrin A1-mediated cell migration is promoted by *C1GALT1* through the activation of EPHA2 in gastric cancer. Immunohistochemical staining of 25 cases of gastric adenocarcinoma revealed that *C1GALT1* protein expression was higher in 80% of the gastric adenocarcinoma tissues than in matched non-tumor gastric tissues, and the low expression levels of *C1GALT1* protein were observed in only 4% of the cases (28). In addition to lymph node metastasis and tumor invasion, high *C1GALT1* expression is often associated with higher histological grade and advanced cancer stage (stage III and IV), and it is an independent prognostic factor of poor survival (28). *C1GALT1* silencing inhibits gastric cancer cell invasion, migration and viability (MKN45 and AGS cells), as well as metastasis and tumor growth (28). *C1GALT1*-knockdown in AGS cells affects multiple functional pathways. Silencing *C1GALT1* decreases phosphorylation and *O*-glycation levels of HER2 and EGFR, as well as inhibiting gastric cancer cell migration (28). Although other pathways are also involved, the viability of cells may be promoted by *C1GALT1* at least in part through the activation of HER2 and EGFR.

Prostate cancer. There is increasing evidence that galectins may interact with abnormal glycosylation and may be associated with cancer progression. Galectin-4 expression is consistently lower in patients with primary prostate cancer compared with in patients with lethal metastatic prostate cancer (102). Galectin-4 activates HER2, EGFR, IGF1 and HER3 receptors in a carbohydrate-dependent manner (102). Tsai *et al* (102) discovered that *C1GALT1* expression in primary tumors is lower than that in castration-resistant prostate cancer. In metastatic prostate cancer samples, it was demonstrated by immunohistochemical analysis that *C1GALT1* was highly expressed in 70% of the samples, and this high expression was closely associated with advanced tumor stage (102). During prostate cancer progression, *C1GALT1* expression is increased and castration resistance is promoted. Notably, metastatic prostate cancer cell lines exhibit high *C1GALT1* gene and protein expression levels (102). The aforementioned findings indicate that there is a close association between tumor malignant transformation and the change of protein *O*-glycosylation and castration resistance. Tumor metastasis may be promoted through interaction with lectin in prostate cancer. Therefore,

the significance of *O*-glycosylation in tumor diagnosis and treatment required to be further explored.

Esophageal cancer. MUC1 is a type I transmembrane mucin, consisting of two subunits, MUC1-N and MUC1-C. High MUC1 expression is associated with a poor prognosis and tumor progression in different types of cancer, making it an oncoprotein (103-106). MUC1 can regulate the WNT signaling pathway by forming intracellular complexes with β-catenin, which in turn can co-activate the expression of cyclin-D1 in the nucleus, ultimately promoting tumorigenesis by allowing cancer cells to avoid apoptotic pathways (107). MUC1 is greatly expressed in esophageal squamous cell carcinoma (ESCC) and ESCC cell migration and invasion can be inhibited by silencing MUC1.

Wang *et al* (108) analyzed MUC1 expression through a large-scale database. MUC1 gene copy number in 102 ESCC tumor samples among 132 ESCC samples was greatly lower than that in 30 cases of adjacent esophageal squamous epithelium. Wang *et al* (108) also analyzed *C1GALT1* expression through the large-scale ONCOMINE database. The average gene copy number of *C1GALT1* in 30 ESCC samples was higher than that in 102 normal esophageal epithelia (108). These data indicate that both MUC1 and *C1GALT1* are abundantly expressed in ESCC. In addition, 7 of the 10 pairs of ESCC samples with high MUC1 *O*-glycosylation had significantly higher expression levels of *C1GALT1* than normal tissues, indicating that *C1GALT1* was positively associated with MUC1 *O*-glycosylation in ESCC (108). MUC1 *O*-glycosylation/*C1GALT1* expression in ESCC without lymph node metastasis was greatly lower in ESCC with lymph node metastasis, and there was a negative association between survival and MUC1 *O*-glycosylation/*C1GALT1* co-expression (108). The aforementioned results suggest that it is possible for MUC1 *O*-glycosylation/*C1GALT1* to be prognostic elements and have diagnostic significance in ESCC, which proposes new insights for targeting MUC1 *O*-glycosylation and *C1GALT1* for inhibiting ESCC metastasis.

Zhang *et al* (51) demonstrated the role of *C1GALT1* expression in the development of radioresistant esophageal cancer. *C1GALT1* protein expression in esophageal cancer tissues was higher than that in adjacent normal tissues. Poor prognosis, lymph node metastasis and TNM staging were associated with upregulation of *C1GALT1* expression. In addition, high levels of *C1GALT1* increased the resistance of esophageal cancer cells to radiation therapy (51). Similarly, Dong *et al* (109) demonstrated that *C1GALT1* could enhance radiation resistance and malignant phenotype of laryngeal cancer cells. Thus, *C1GALT1* is very important in carcinogenic resistance to radiotherapy.

Cholangiocarcinoma. *C1GALT1* serves a role in the development of cholangiocarcinoma. Cholangiocarcinoma tissues have higher *C1GALT1* expression than normal bile ducts (110). Additionally, elevated *C1GALT1* expression in cancer tissues is associated with advanced cell grade, larger tumor size and tumor stage (110). The inhibition of *C1GALT1* can significantly inhibit the viability, migration and invasion of cholangiocarcinoma cells, whereas overexpression of *C1GALT1* can promote these abilities (111). This indicates that *C1GALT1* is critical for cancer progression in cholangiocarcinoma.

Head and neck cancer. Lin *et al* (13) demonstrated that *C1GALT1* expression is upregulated in head and neck squamous cell carcinoma (HNSCC), and high *C1GALT1* expression is associated with poor clinicopathological characteristics. In addition, *C1GALT1* can modify the O-glycans on the EGFR. Previous studies have revealed that O-glycan modification can influence the behavior of cancer cells and their signal transduction pathway (27,83,85,112). Phosphorylation RTK array assay in HNSCC indicated that the phosphorylation of MET and EGFR is mostly decreased by *C1GALT1* knockout or knockdown (13). The EGFR signaling pathway is important in the invasion and survival of tumor cells in HNSCC (113). Lin *et al* (13) provided evidence via mass spectrometry that EGFR has GalNAc type O-glycans, indicating that *C1GALT1* can modify EGFR. Subsequently, SAS cells overexpressing *C1GALT1* were constructed. The EGF-induced EGFR phosphorylation at Y1068 was improved by *C1GALT1* overexpression, and HNSCC cell invasion, migration and activity was also improved (13). EGF-EGFR binding affinity was decreased by the knockout of *C1GALT1* in SAS cells, and the EGFR signaling pathway was inhibited. Additionally, the invasion, migration and viability of SAS cells treated with erlotinib, an EGFR tyrosine kinase inhibitor, were reversed (13). The aforementioned results indicated that *C1GALT1* may change the glycosylation of EGFR. In HNSCC cells, *C1GALT1* enhances the binding affinity to the EGF ligand, as well as phosphorylation of EGFR, increasing the malignant phenotype.

Ovarian cancer. Immature truncated O-glycans have usually been detected in the ovarian cancer cells of human beings, and evidence indicates that these changes in glycosylation expression can contribute to various types of cancer, including colon and ovarian cancer, which usually express short O-glycans (114,115).

Chou *et al* (116) evaluated the prognostic value of *C1GALT1* expression through analysis of patients with ovarian cancer in a public database, generating survival curves of each patient. In all patients with ovarian cancer followed for 20 years, a low overall survival rate was associated with high *C1GALT1* expression (hazard ratio, 1.19; 95% CI, 1.04-1.37; P=0.014) (116). These results indicate that targeting *C1GALT1* may be a promising strategy for ovarian cancer (116). Further research on *C1GALT1* is essential for an improved understanding of the occurrence of ovarian cancer.

Overall, the aforementioned findings indicate that *C1GALT1* promotes tumor development. However, in other cases, *C1GALT1* may also have a tumor-suppressing effect.

4. *C1GALT1* as a tumor suppressor

In the aforementioned types of tumor, *C1GALT1* expression is usually upregulated during tumorigenesis. However, the expression levels of *C1GALT1* in colorectal and pancreatic cancer are different from the aforementioned types of tumor.

Pancreatic cancer. The loss of *C1GALT1* in mice caused increased truncated O-glycan expression, which caused the

metastasis of pancreatic ductal adenocarcinoma (PDAC) (30). Genetically engineered KPC and KPCC mice models were created by breeding $Kras^{G12D/+}$, Pdx1-Cre and $LSLTrp53^{R172H/+}$ with $C1galt1^{loxP/loxP}$ (30). The KPC pattern was adopted to create pancreas-specific *C1GALT1* depletion (KPCC mice) and monitor pancreatic tumor progression and growth in these mice (30). The survival time of KPCC mice (median, 102 days) was longer compared with that of KPC mice (median, 200 days), and KPCC mice developed early pancreatic intraepithelial neoplasia at 3 weeks, PDAC at 5 weeks and metastases at 10 weeks compared with KPC mice (30). Moreover, metastases to distant organs in KPC mice were observed after 20 weeks (30). Compared with other PDAC animal patterns, KPCC is considered to be the predominant PDAC mouse pattern to present primary metastasis (117,118). Compared with KPC mice, pancreatic tumors in KPCC mice have been considered to be more metastatic and aggressive, and Tn production is increased, while the number of stromata is decreased (30). Pathological analysis of tumor tissues has shown that most KPCC tumors are poorly differentiated or undifferentiated, while most KPC animals have moderate to highly differentiated tumors (30). It is worth noting that when *C1GALT1* is conditionally inactivated without the background of carcinogenic mutations, the pancreas appears normal (30). This indicates that loss of *C1GALT1* alone does not lead to the formation of PDAC. Loss of *C1GALT1* is associated with p53 and KRAS mutations leading to faster progression of PDAC (30).

According to experiments performed in cell lines, human PDAC cells with *C1GALT1* gene knockout have greatly developed MUC16 abnormal glycosylation, tumorigenicity and invasion, proliferation and increased expression of Tn carbohydrate antigen compared with a control group (PDAC cells without *C1GALT1*-knockout) (30). Growth factor receptor activation, such as HER2 and EGFR, as well as activation of downstream effectors, such as Akt and focal adhesion kinase (FAK) proteins, is promoted, and MUC16 activates metastasis signals and interacts with FAK (119). PDAC cell migration is induced by the activated signals of Akt and FAK, which may possibly explain the increased migration of *C1GALT1*-knockout cells (30). It is necessary to conduct further research on this topic in the future.

Colorectal cancer (CRC). The expression of Tn antigen is associated with various types of cancer metastasis and progression (120). For example, immature truncated O-glycans (like the Tn antigen) can usually be detected in human CRC (121). Bergstrom *et al* (122) argued that there was no association between cancer progression and Tn antigen by adopting a CRC murine pattern. Instead, intestinal inflammation has been shown to lead to eventual tumorigenesis rather than abnormal *O*-glycosylation (122). Mice lacking core 1-derived O-glycans (IECC1galt1-/-) developed spontaneous colitis. Between 18 and 24 months, ~90% of mice developed colon tumors, with an average of 3 tumors, of various sizes (122). *In vivo* analysis revealed that Tn exposure itself did not significantly promote colon inflammation and tumorigenesis. Thus, the incidence of carcinogenesis in patients who have UC may be decreased by core inflammatory pathway inhibition. Nevertheless, Dong *et al* (123) indicated that forced knockout of *C1GALT1* in HCT116 cells significantly induced Tn antigen expression and contributed to metastasis and progression of CRC. It seems that Tn antigen can be adopted as an underlying target of therapeutic intervention (124,125). T-synthase deficiency in CRC cells may lead to the activation of the EMT signaling pathway. EMT is important in cancer progression (126,127). Knockout of *C1GALT1* in HCT116 cells can greatly enhance the adhesion and proliferation of cells and induce Tn antigen expression (123). Moreover, E-cadherin (a typical epithelial marker) was markedly decreased in *C1GALT1*-knockout HCT 116 cells, accompanied by an enhanced expression of mesenchymal markers including snail and fibronectin (123). These observations indicate that T-synthase deficiency can induce abnormal *O*-glycosylation in cells, subsequently promoting carcinogenesis by activating the EMT process (123).

The aforementioned studies reported the dual role of *C1GALT1* in cancer (carcinogenesis and cancer suppression), and the association between this gene and several molecules and signaling pathways has been explored. This may provide new therapeutic strategies for cancer treatment. Table I shows the role of *C1GALT1* in different types of cancer.

5. Cosmc and integrin β1

Cosmc is the molecular partner of T-synthase, helping T-synthetase to fold correctly in the ER (128). This chaperone is encoded by Cosmc in the X chromosome (human Xq24, mouse Is Xc3). Cosmc is located in the ER. The newly synthesized T-synthase needs Cosmc to avoid incorrect folding, aggregation and degradation. Human Cosmc is a type II transmembrane protein with 318 amino acids (36). It has a short N-terminal domain, a transmembrane domain and a large C-terminal domain in the cytoplasm, which can independently act as a molecular chaperone for T-synthase (36). Cosmc protein itself does not possess galactosyltransferase activity. However, the expression of functional T-synthase must be accompanied by the presence of Cosmc (129). There is 26% homology in amino acid sequence between human T-synthase and human Cosmc, indicating that they are from the same ancestor (36,129). In humans, Cosmc and T-synthase are universally expressed and work cooperatively, but their expression levels vary by tissue or cell type (15,130). Zeng *et al* (131) reported that the promoter structures of Cosmc and T-synthase are similar. The CpG islands in the 5' flanking regions of human Cosmc and T-synthase are gene promoters, and they each contain two SP1/3 binding sites (131). Chromatin immunoprecipitation analysis and site-directed mutagenesis analysis of any SP1/3 site confirmed the important role of the SP1/3 sequence in regulating these two genes (131). In patients with Tn syndrome lacking functional Cosmc, T-synthase activity is completely absent, indicating that Cosmc is an important partner in the formation of active T-synthase (131). Upon lack of functional Cosmc, T-synthase will be reversely transported from the ER back to the cytoplasm, ubiquitinated and degraded in a 26S proteasome-dependent manner (131).

Lack of Cosmc is fatal to mice embryos (37,132). Knockout of Cosmc or T-synthase in mice causes the expression of Tn antigen and embryonic lethality (20,132). Wang *et al* (132) found that mice with obvious absence of Cosmc have lung and gastrointestinal bleeding, chylous ascites and growth retardation, and this state is similar to the conditional T-synthase

Table I. Role of C1GALT1 in different types of cancer.

First author, year	Cell lines	Model	Effects	Expression in cancer	Type of cancer	(Refs.)
Wang *et al*, 2020	HA22T, PLC5	*In vitro*, *in vivo*, human tissue	Regulation of the O-glycosylation level of the MET protein activates the HGF signaling pathway	Upregulation	Hepatocellular carcinoma	(87)
Zhang *et al*, 2018	ECa109	*In vitro*, *in vivo*, human tissue	Radiation resistance is inhibited by glycosylation of the modifier β1 integrin	Upregulation	Esophageal cancer	(51)
Lee *et al*, 2020	AGS	*In vitro*, *in vivo*, human tissue	Activation of EPHA2-promoted cell migration mediated by soluble Ephrin A1	Upregulation	Gastric cancer	(28)
Huang *et al*, 2015	HUCCT1	*In vitro*, human tissue	C1GALT1-knockout inhibits the malignant behavior of bile duct cancer cells	Upregulation	Cholangiocarcinoma	(111)
Lin *et al*, 2018	OEC-M1, FaDu	*In vitro*, *in vivo*, human tissue	C1GALT1-knockdown blocks O-glycan extension on EGFR and inhibits EGFR signal transduction	Upregulation	Head and neck squamous cell carcinoma	(13)
Chou *et al*, 2017	ES-2	*In vitro*, human tissue	Regulates the expression of multiple genes associated with tumor stem cells in ovarian cancer cells	Upregulation	Ovarian cancer	(116)
Chugh *et al*, 2018	T3M4	*In vitro*, *in vivo*, human tissue	C1GALT1-knockdown promotes the occurrence and metastasis of pancreatic adenocarcinoma	Downregulation	Pancreatic ductal adenocarcinoma	(30)

C1GALT1, core 1 synthase glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; EPH, ephrin.

deletion in hematopoietic cells and endothelial cells observed in mice. These findings indicate that the lack of O-glycans in endothelial cells can lead to misconnection of blood/lymphatic vessels, and that T-synthase and its molecular chaperone Cosmc are both necessary for the proper development of blood vessels (21).

Acquired mutations in Cosmc are associated with a number of diseases, such as IgA nephropathy and Tn syndrome. Some of the Cosmc gene has genetic deletion in invasive human melanoma LOX cells, while point mutations exist in other cell lines, causing Cosmc inactivation and elevating Tn antigen expression (64). For example, human cervical cancer cells exhibit Cosmc deletion (65). In pancreatic cancer, epigenetic silencing by Cosmc promoter methylation leads to inactivation of T-synthase, accompanied by abnormal *O*-glycosylation (133). Additionally, Cosmc point mutations are found in several epithelial samples of patients with UC (75), but it is unclear if this is associated with an increased risk of colon cancer.

Cosmc is required for the functional expression of T-synthase. The expression of Tn antigen and T-synthase activity loss is a result of human Cosmc loss, which is associated with several diseases (60,61,63,66,134-136), such as Tn syndrome (61), IgAN (137) and human tumor (68). Thus, although these outcomes do not elucidate the Cosmc chaperone impact, the role of Cosmc in *O*-glycosylation seems to specifically rely on T-synthase. The proper function of T-synthase requires the molecular chaperone Cosmc, and the integrin β1 subunit may be involved in mediating these functions.

C1GALT1 can regulate the activity and glycosylation of integrin β1 (29). Integrin β1 belongs to the integrin family, which consists of transmembrane proteins. It can transduce changes in the extracellular mechanical state and chemical environment of the cell, which can lead to cytoskeletal changes. It participates in a wide range of functional activities, such as cell proliferation, invasion, adhesion and inflammation (138). According to previous studies, there is a close association between integrin β1 and improvement in therapeutic drug resistance in different hematopoietic malignancies and solid tumors, and drug resistance of tumors is mediated by integrin β1 at the cellular level (139,140). A study has indicated that blocking integrin β1 inhibits breast cancer cell proliferation and induces apoptosis (141). Integrin β1 has a close association with TNM grade and tumor size in liver cancer (142). High expression levels of integrin β1 are associated with worse survival in patients with liver cancer (143). Moreover, a previous study has indicated that C1GALT1 induces hepatocellular carcinoma cell adhesion to extracellular stroma proteins via integrin β1, as well as inducing cancer cell migration and invasion (29). C1GALT1 regulates integrin β1 activity as well as its downstream signaling through the modification of the O-glycan on integrin β1 (29,144). The interaction between MET and integrin in the regulation of development, immunity and invasion of cancer cells has been previously reported (145-147). Since HGF-triggered cell proliferation is enhanced by C1GALT1 via MET, it is a reasonable assumption that the signaling pathways of MET and integrin β1 promote C1GALT1-mediated HCC malignancies synergistically. These findings further prove that mucin-type *O*-glycosylation is important in regulating cancer malignancies, indicating that C1GALT1 may be a promising therapeutic candidate.

Targeting integrin β1 with inhibitory antibodies can increase the sensitivity of hepatocellular carcinoma cells to radiation (148). Moreover, the inhibition of integrin β1 using antibodies or siRNAs causes dose-dependent radiation sensitization of head and neck cancer cells (149). The downregulation of integrin β1 in laryngeal carcinoma can inhibit glycosylation-mediated radiation resistance (123). In esophageal cancer, C1GALT1 can regulate the signaling pathway of the downstream FAK and modify the O-glycan structure on integrin β1 (51). Moreover, in esophageal cancer cells, integrin β1 blocking antibodies and FAK inhibitors can enhance radiation-induced apoptosis (51).

In conclusion, the aforementioned results indicate that C1GALT1 and integrin β1 signaling pathways can synergistically promote intrinsic radiation resistance mediated by glycosylation, although the detailed mechanism of this phenomenon remains elusive.

6. Itraconazole, an inhibitor of C1GALT1

Itraconazole is a common antifungal drug with anticancer effects. Itraconazole has been beneficial in patients with ovarian cancer, recurrent non-small cell lung cancer, prostate cancer and other types of cancer, either as a single drug or in combination therapy in clinical trials (150-153). Lin *et al* (13) proposed itraconazole as a new important *C1GALT1* inhibitor in head and neck cancer. Lin *et al* (13) screened the ZINC database for compounds that could bind to the *C1GALT1* protein. A total of seven drugs were found not to be standard anticancer treatments and had fewer side effects. Only itraconazole significantly increased the expression of Tn antigens on several cell surfaces (13). At the same time, itraconazole significantly decreased the protein expression levels of *C1GALT1*, while mRNA expression was not significantly affected, suggesting that itraconazole may affect the protein level of *C1GALT1* through post-translational modification (13). In general, *C1GALT1* protein folding errors are transported to the proteasome and then degraded (154). The proteasome degradation pathway involves ubiquitination, and itraconazole increases ubiquitinated *C1GALT1*. The results of the cell thermal displacement analysis revealed that when using itraconazole to treat SAS and OEC-M1 cells, the melting temperature of *C1GALT1* decreased, and itraconazole decreased the protein expression levels of *C1GALT1* in a dose-dependent manner at a constant melting temperature (13). Vicia villosa agglutinin pull-down tests indicated that itraconazole increased the Tn antigen on EGFR (13). SAS cells that overexpressed *C1GALT1* and OEC-M1 cells with inhibited *C1GALT1* were injected in a mouse xenotransplantation model (13). The tumor growth rate and volume of SAS cells increased significantly, while the tumor growth of OEC-M1 cells decreased significantly (13). *C1GALT1*-mediated tumor growth was partially reversed by itraconazole in SAS cells (13). The aforementioned results indicate that *C1GALT1* greatly influences HNSCC, and silencing *C1GALT1* may potentially be an underlying treatment for tumors (13). Although *C1GALT1* expression in mice is partially inhibited by itraconazole, targeting *C1GALT1* via genetic molecular pathways can have great therapeutic potential for cancer treatment.

7. Conclusion

Glycosylation is a common, complex and diverse post-translational modification. This diverse polysaccharide has a wide scope of biological functions. Mammalian angiogenesis, platelet production and kidney development are inseparable from

O-glycosylation. The orderly construction of sugar molecules in normal cells involves substrate-specific glycosyltransferases (51,155). *C1GALT1* and glycosylation are essential for normal development. Impaired T-synthase activity has been associated with different types of human diseases, including inflammatory or immune-mediated diseases, and cancer. The present review highlighted the relevance of *C1GALT1* in the pathogenesis of IgAN, Tn syndrome, IBD and various types of cancer.

The change in glycosylation was discovered in a malignant transformation 60 years ago, and this change is considered to be one of the hallmarks of human cancer pathogenesis (156). Abnormal *O*-glycosylation, which found in various types of tumor, is very important in metastasis progression (83,157-159). The abnormal *O*-glycosylation of proteins on malignant tumor cell surface participates in different steps of tumor progression and regulates intercellular and intracellular signal transduction, thus inducing angiogenesis, EMT, metastasis and cell proliferation (157,160). The protein encoded by the *C1GALT1* gene is a key mucin-type O-glycosyltransferase located in the Golgi apparatus (17). Galactose transfers to Tn antigen with its molecular chaperone Cosmc, forming Galβ1-3GalNAcαSer/Thr structure (T antigen, core 1 structure) (83). In cases of hepatocellular carcinoma and cholangiocarcinoma, *C1GALT1* expression is usually upregulated during tumorigenesis (27,109). Additionally, C1GALT1 silencing can inhibit cancer cell migration, invasion and proliferation, which inhibits metastasis and tumor growth (27,28). The *C1GALT1* and integrin β1 signaling pathways can synergistically promote glycosylation-mediated intrinsic radiation resistance (149). Abnormal *O*-glycosylation is involved in the process of EMT (123). In addition, changes in *C1GALT1* expression can cause short O-glycan expression in different types of cancer, which leads to cancer progression (120). *C1GALT1* expression in mice is inhibited partially by itraconazole (13). Targeting *C1GALT1* via genetic molecular pathways can have great therapeutic potential for cancer treatment. On the contrary, *C1GALT1* acts as a tumor suppressor gene in colon and pancreatic cancer (30,123). Using samples from different sources or at different tumor stages may contribute to the observed duality in the *C1GALT1* function, rendering the true role of this gene still elusive.

In conclusion, it is of great necessity to implement further studies for exploring the role of *C1GALT1* and *O*-glycosylation, as well as its molecular chaperone Cosmc, and their interaction with different *C1GALT1* targets, such as integrin β1, in the clinical setting. Future studies will help improve the understanding of certain pathologies and find new ways to treat and prevent disease in the future.

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Competing interests

The authors declare that they have no competing interests.

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