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UDP-GalNAc: polypeptide α-N-acetygalactosaminyltransferase 2 Localized on Both *cis* and *trans* Side of Golgi Stacks in SGC7901 Cells*

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Abstract Uridine diphosphate (UDP)-GalNAc：polypeptide N-acetylgalactosaminyltransferase (ppGalNAcT) catalyzes the initial step in mucin type O-glycosylation in the Golgi apparatus. Here generation and characterization of a polyclonal antibody to human ppGalNAcT2 were described. The subcellular location of ppGalNAcT2 in SGC7901 cell line was investigated using Western blot analysis of fractionated cell extracts and confocal microscopy with this antibody and two Golgi markers: Golgi SNARE (soluble N-ethylmalemide-sensitive factor attachment protein receptor) of 28 ku (GS28) and *trans*-Golgi network (TGN) 38, markers for the *cis*- and *trans*-Golgi apparatus, respectively. Morphometric analyses indicated that ~ 60% of the ppGalNAcT2 signal colocalized with the GS28, while ~ 36% of the *cis*-Golgi marker colocalized with the ppGalNAcT2. Approximately 34% of the ppGalNAcT2 signal colocalized with the TGN38, whereas 38% of the *trans*-Golgi marker colocalized with the ppGalNAcT2. The results provide unequivocal evidence for the location of ppGalNAcT2 within the Golgi apparatus, and further highlight the importance of this organelle in the initiation of O-linked glycosylation.

Key words ppGalNAcT, Golgi apparatus, O-linked glycosylation, localization
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Glycosyltransferases comprise a large group of enzymes involved in the synthesis of complex carbohydrates of glycoproteins, glycolipids and glycosaminoglycans. The enzymes are membrane bound and localized in the endoplasmic reticulum (ER) or Golgi complex[1]. Biochemical and ultrastructural studies indicate that glycosyltransferases partially segregate into distinct compartments within the secretory pathway. In general, early acting Golgi glycosyltransferases are located in ER, *cis* and medial compartments of Golgi whereas late acting enzymes are mainly found in the *trans*-Golgi cisternae or the *trans* Golgi network (TGN)[2]. However, other reports suggested a less rigid compartmentalization of glycosyltransferases, since enzymes such as β1,2 N-acetylglucosaminyltransferase Ⅰ and α 1,2-mannosidase Ⅱ have been observed to be present in more than one Golgi compartment[3, 4] and variations from the distribution of a given enzyme have been reported to occur among different cell types[5]. Finally, there are reports about cell surface localizations[6] and recycle from the Golgi to the ER of glycosyltransferases[7, 8].

The first committed step of carbohydrate addition to mucin-type glycoproteins is catalyzed by a family of UDP-GalNAc：polypeptide α-N-acetylgalactosaminyltransferases, ppGalNAcTs (EC 2.4.1.41), yielding the Tn antigen (GalNac-α-1-O-Ser/Thr). Subsequent elongation of the Tn structure yields an array of 8 distinct "core" glycans that can be further modified by many of the glycosyltransferases resident in the Golgi. Compared with N-linked glycosylation of proteins, which is initiated cotranslationally in the ER, the intracellular site of O-glycan initiation remains a matter of debate. Various compartments of the secretory pathway ranging from the ER[9] and the ER to Golgi

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intermediate compartment (ERGIC)[10] to the *cis* side of the Golgi apparatus[11, 12] have been implicated in the initiation of O-glycosylation. These conflicting results may be explained by subcellular localization of ppGalNAcTs that may contain as many as 24 family members in mammals[13]. In human fifteen subtypes or isoforms (ppGalNAcT1～15) have been identified and characterized[14]. Among them, ppGalNAcT2 was found widely distributed and expressed constitutively in most human tissues[15], therefore, we studied the localization of ppGalNAcT2 to probe the initiation site of O-glycosylation.

In this study, we investigated the intracellular localization of ppGalNAcT2 by immunocytochemical methods. Since protein-specific antibodies for glycosyltransferases remain the tools of choice to address problems related to cellular localization, we used the strategy of expressing the glycosyltransferase antigen as a glutathione S-transferase (GST)-T2 fusion-protein in BL21 to obtain non-glycosylated peptide antigens for the induction of protein specific polyclonal antisera[16] since presence of carbohydrate specific antibodies could cause misleading results[17]. The characterization of this newly developed antibody proved its feasibility for subcellular localization. We used the antibody for immunocytochemistry to probe the location of ppGalNAcT2 within SGC7901 cells. Our study demonstrate the presence of ppGalNAcT2 in both *cis* and *trans* Golgi stack suggesting that O-glycosylation may be initiated beyond the *cis* Golgi.

1 Materials and methods

1.1 Materials

SGC7901 cell line were obtained from Shanghai Institute of Cell Biology. Fetal calf serum (FCS), RPMI-1640 mediums were purchased from GIBCO/BRL. Taq E polymerase, dNTP mixture and restriction enzymes were purchased from MBI Company. TRIzol, RNase inhibitor, Superscript Ⅱ RNase H reverse transcriptase and pUC Mix DNA marker were from Invitrogen Company. All primers were synthesized by Invitrogen Company (Shanghai). Antibody to Golgi SNARE (soluble N-ethylamide-sensitive factor attachment protein receptor) of 28 ku (GS28) and *trans*-Golgi network (TGN) 38 were purchased from Acris. The other reagents were commercially available in China.

1.2 Construction of ppGalNAcT2 vectors

Total RNA from the SGC7901 cell line was isolated by TRIzol. Double stranded cDNA was synthesized by reverse transcription using oligo dT primers. Oligonucleotides corresponding to the flanking regions of the full length of the ppGalNAcT2 sequence were used as specific primers for PCR amplification (5′ ACCACGGCTTGAAAGTACGGT-GGCCAGACTTT 3′ and 5′ ACCACCGGTCTA-CTGCTGCAGGTTGAGCGTGAA 3′). This PCR product was then ligated into pUCmT vector. For expression of GST-ppGalNAcT2-lectin fusion protein (T2-FP), we subclone a truncated form of ppGalNAcT2 into the expression vector pGEX-5x-3 with the primer 5′ TTAGAATTCCGACCATCA-GGA 3′ and 5′ GACTCGAGAGTGAACTTCCA 3′, which contain *Eco*R Ⅰ and *Xho*l Ⅰ restriction site.

1.3 Expression and purification of T2-FP

Recombinant plasmid vector was transformed into BL21. After induction of protein expression by IPTG, cells were harvested and lysed, T2-FP was obtained by fractionation on a 12% SDS-polyacrylamide gel, excision of the T2-FP band from the gel and elution.

1.4 Induction of antibodies to T2-FP and their purification

Purified antigen (0.5 mg) of emulsified with complete Freund adjuvant (Sigma) was subcutaneously injected into rabbit (New Zealand White) followed by three boost injections with incomplete adjuvant every week. For construction of the affinity columns 1 mg of T2-FP and 1 mg of GST were immobilized on Affi-Gel (Bio-Rad), respectively. The whole purification process was according to the user's manual.

1.5 Preparation of subcellular fractions

SGC7901 cells (human gastric cancer cell line) were washed with ice-cold HME buffer (10 mmol/L HEPES, 250 mmol/L mannitol, 0.5 mmol/L EDTA, pH 7.4), resuspended in 5 volumes of ice-cold HME buffer containing 0.1 mmol/L phenylmethylsulfonyl fluoride, and Dounce homogenized with 10 gentle strokes. Nuclei were pelleted at 1 500 *g*, followed by a spin at 10 000 *g* (10 min, 4℃). The 10K pellets were resuspended in HME buffer. The 10K supernatant (after saving an aliquot) was overlaid on a 20% sucrose cushion and further centrifuged at 100 000 *g* (60 min, 4℃). The 100K pellets were resuspended in HME. The 100K supernatant was also saved for Immunoblot analysis.

1.6 Immunoblot analysis

For immunoblot analysis, proteins from cell

lysates and purified proteins were separated by SDS-PAGE on a 12% gel and electrotransferred to a PVDF membrane. The membrane was blocked with blocking buffer (1% milk powder, TBST: 15 mmol/L NaCl, 5 mmol/L Tris, 0.1% Tween-20 pH 7.5) at 4℃ overnight. The blot was then probed 1 h with suitably diluted first antibodies, washed three times 20 min with TBST, incubated 1 h with horseradish peroxidase (HRP)-conjugated anti rabbit IgG 1∶1 000 diluted in blocking buffer, and washed again three times with TBST. The blot was developed with the enhanced chemiluminescence (ECL) detection kit (Amersham).

1.7 Immunocytochemistry

Cells were cultured at 37℃ on glass coverslips to subconfluency. Before staining, they were washed with phosphate-buffered saline (PBS), fixed for 10 min by 4% paraformaldehyde at room temperature. The cells were permeabilized with 0.1% Tween-20 in intracellular buffer (75 mmol/L potassium acetate, 2.5 mmol/L magnesium acetate, 1.8 mmol/L calcium chloride, and 25 mmol/L HEPES buffer pH 7.2) with 0.4% BSA for 30 min at room temperature. All subsequent steps, including the washes, were performed in an intracellular buffer containing 0.1% Tween-20 and 0.4% BSA. The cells were incubated overnight with primary antibodies at 4℃ and then rinsed three times for 10 min. The appropriate secondary antibodies were applied for 2 h at room temperature. Primary antibodies were used at the following dilutions: anti-T2, 1∶500 (v/v); anti-GS28, 1∶100(v/v) and anti-TGN38, 1∶100(v/v). Fluorescent secondary antibodies were used at the following dilutions: Cy3-conjugated anti-rabbit IgG, 1∶1 000 (v/v) and FITC-coupled anti mouse IgG, 1∶100 (v/v). In double-labeling experiments, the two primary antibodies were applied simultaneously, followed by a mixture of the two secondary antibodies.

1.8 Confocal microscopy analysis

Images were collected and analyzed on a Leica TCS SP2 confocal laser scanning inverted microscope. The 488 line of an argon laser or the 543 line of an HeNe laser were used for excitation of fluorescein and Cy3-labeled secondary antibodies, respectively. Colocalization of the two fluorophores was computed as a percent of a one-color pixel that overlapped with a separate-color pixel. Analysis of colocalization was done using MetaMorph 5.0, with the threshold set to display the brightest 95% of positive pixels. For display, images were converted into TIFF format and processed using Adobe Photoshop Software.

2 Results

2.1 Bioinformatics analysis and antigenic region selection of T2

The ppGalNAcT family is unique among glycosyltransferases, containing both catalytic and lectin domains that have shown to be closely associated[18~20](Figure 1a*A*). According to the report of Fritz[20], among this family member, catalytic domain is more conserved and homologous than lectin domain and other regions. And multiple sequence alignment of this family (Figure 1b) indicating the sequence of ppGalNAcT2 lectin domain shows lower similarity in homology from those of the other members. Antigenic index analysis using DNAStar Protean software suggests that ppGalNAcT2 has multitude potential antigen eptitope throughout the whole molecular (Figure 1a*B*). Considering reports which have shown that immunogenicity of glycosyltransferases is associated with the least conserved domain, i.e., the stem region [16], we decided to raise antibodies to the region containing lectin domain (residues 441 to 571) as a GST linked fusion-protein.

2.2 Construction of ppGalNAcT2-lectin expression vector (pGEX-5x-3-T2-lectin) and expression of the GST-ppGalNAc-T2-lectin fusion-protein (T2-FP)

Total RNA was isolated from SGC7901 cells and the mRNA fraction was reverse transcribed to cDNA by oligo dT primers. The ppGalNAcT2 sequence was amplified by PCR using specific primers flanking the full length of the coding region (Figure 2a). The PCR product was used for subsequent cloning into pUCm T vector. Residues 441～571 of T2 encoding the entire lectin domain were subcloned into the expression vector pGEX-5x-3. Thus, the sequence coding for the most conserved regions was eliminated (Figure 1a*D*).

The GST-T2-lectin fusion-protein (T2-FP) was produced in BL21 following transformation and induction by IPTG. This heterologously expressed T2-FP had a molecular mass of about 40 ku(Figure 2b). After separation of the proteins by SDS-PAGE, the band representing the fusion-protein was cut out, and the protein was eluted and analyzed for purity (Figure 2b). Electrophoretically pure fractions were then used for the immunization of rabbit as described in **Materials and methods**.

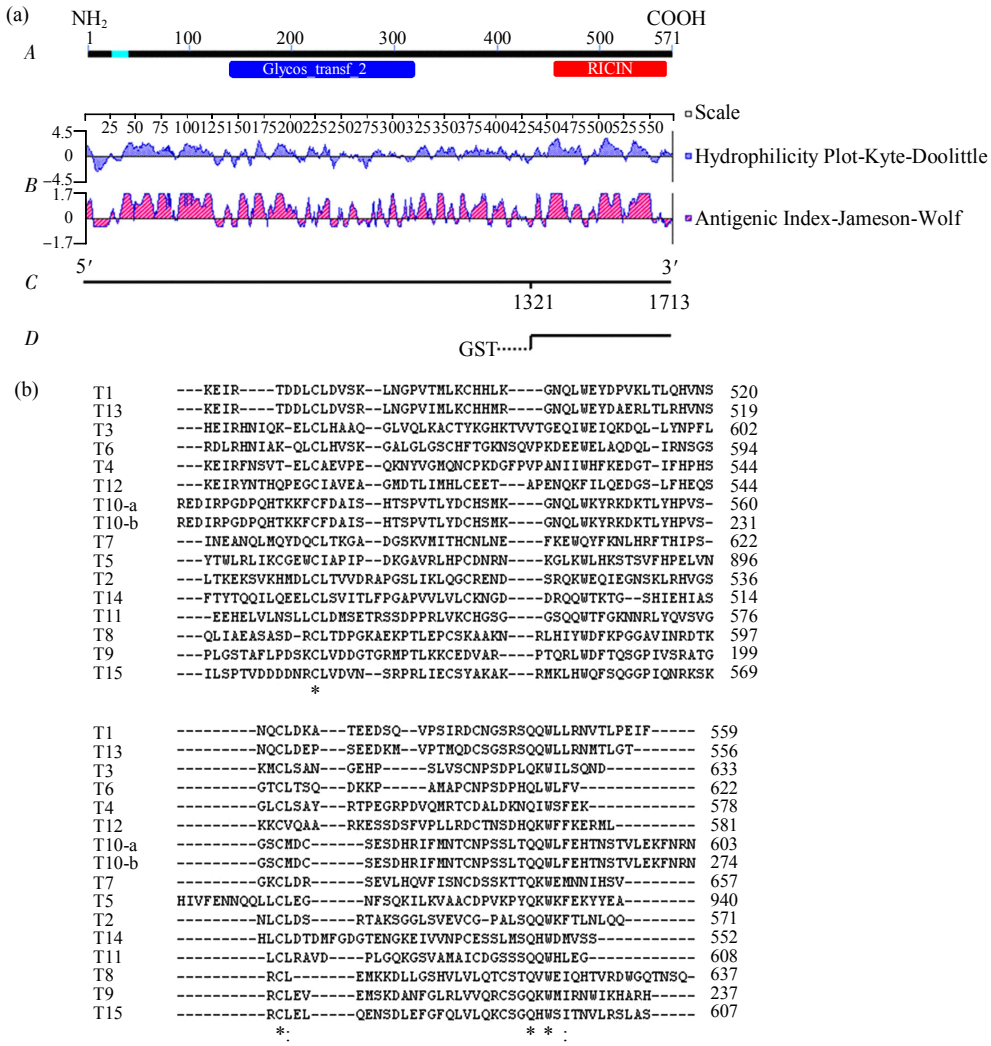

Fig. 1　Schematic view of ppGalNAcT2(a) and Multiple sequence alignment (ClustalW) of 15 human ppGalNAcTs (b)

A: Scheme of the ppGalNAcT2 protein sequence with catalytic domain and lectin domain (NCBI rpsblast). *B*: Scheme of hydrophilicity and antigenic index of ppGalNAcT2 (DNAStar Protein). *C*: ppGalNAcT2 DNA sequence of the PCR-cloned form from SGC7901 cells. *D*: DNA sequence encoding truncated (residues 441 to 571) ppGalNAcT2 that was introduced into the expression vector which was used for the production of GST fusion-protein. Only sequences of the lectin domains are shown in (b).

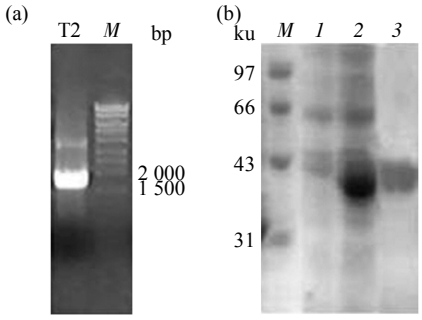

Fig. 2　Cloning of ppGalNAcT2 and expression of T2-FP

(a) PCR product of ppGalNAcT2. The PCR product is consistent with theoretical molecular mass 1 713 bp, which is confirmed by DNA sequencing. DNA was electrophoresed in a 1.5% agarose gel. The gel was stained with EB. (b) SDS-PAGE analysis of the recombinant protein. Slot *1* was IPTG (–), Slot *2* was IPTG(+), slot *3* was the purified protein. The fusion-protein has a molecular mass of about 40 ku. Proteins were separated in 12% separating gel and stained with Coomassie Brilliant Blue R-250.

2.3　Characterization of the antibodies against human ppGalNAcT2

The polyclonal antibodies against human T2-FP (anti-T2-FP) obtained by immunizing rabbit with this heterologously expressed T2-FP were characterized first by ELISA. The antiserum had a titer of about 1∶6 400 toward the coated T2-FP, whereas binding of the preimmune serum was negligible at the same dilution (not shown). Since use of this antiserum to localize ppGalNAcT2 in cells was intended, we prepared a fraction of affinity purified antibodies devoid of antibodies specific for GST. The purification scheme (Figure 3) involved two affinity purification steps, first on the fusion-protein column (yielding anti-T2-FP) followed by a GST column (yielding

anti-T2). Antibodies retained on the GST column were designated anti-GST. As shown in Figure 4, these antibodies were tested by immunoblotting for reactivity toward T2-FP (slots *1*, *3*), *E. coli* GST (slots *2*, *4*). The anti-T2-FP (Figure 4a) reacted with both antigens. The anti-T2 (Figure 4b) reacted with the fusion-protein (slot *3*), but not with GST (slot *4*), indicating efficient removal of these antibodies. Immunoblots (Figure 4, slot *5*, *6*) using the anti-T2 fraction on mock and pcDNA3.1-T2 transfected SGC7901 cell lysate revealed one band at 70 ku, which may correspond to a mature and posttranslationally modified, but denatured form of ppGalNAcT2. Blotting (Figure 4c) using preimmune serum on mock and transfectant were used as control, which showed no significant signal. These data show this antibody is suitable for immunocytochemical applications.

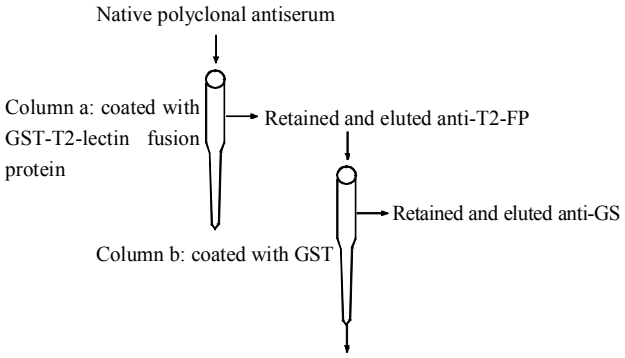

Fig. 3 Scheme of the affinity purification steps of anti-T2-FP antisera

Antibodies retained on the fusion-protein column (anti-T2-FP) were subsequently affinity purified on a GST column. The flow through of this column (anti-T2) was specific for the ppGalNAcT2 part of the fusion-protein.

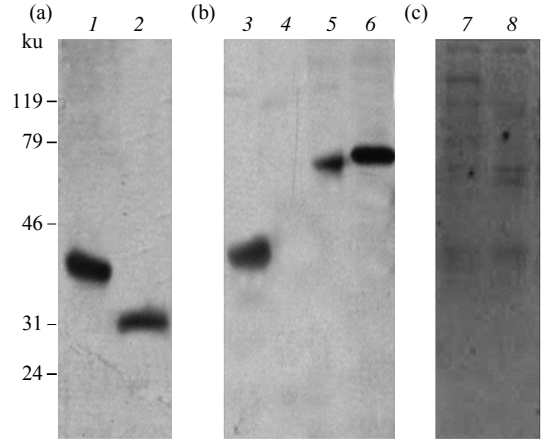

Fig. 4 Immunoblots using different antibody fractions

Anti-T2-FP (a), anti-T2 (b) and preimmune serum(c) were probed at the same dilution 1∶5 000 (*v*/*v*) (30 μg/L) for crossreactivity with fusion-protein (slots *1*, *3*), GST (slots *2*, *4*) and lysate of mock (slot *5*, *7*) and ppGalNAcT2 transfected (slot *6*, *8*) SGC7901 cells.

2.4 Subcellular localization of ppGalNAcT2 by biochemical subcellular fractionation

To characterize ppGalNAcT2 localization, we performed biochemical fractionation of SGC7901 cell lysates. After the separation of nuclei and post-nuclear supernatant (PNS), heavy membranes including mitochondria, lysosomes, peroxisomes and ER membranes were pelleted by low speed centrifugation (10 000 *g*) [21]. ppGalNAcT2 was found in the supernatant (10K supernatant, Figure 5b). The 10K supernatant was further fractionated by high speed centrifugation (100 000 *g*) to pellet light membranes, including some ER and Golgi apparatus membranes. We also found that ppGalNAcT2 was in the 100K pellet but not the 100K supernatant fraction (Figure 5b), which suggest that ppGalNAcT2 is associated with the Golgi apparatus. As a control for the fractionation procedure, proteins with a well-known subcellular localization were also tested. Figure 5a also shows that the cytoplasmic protein α-tubulin, a component of the microtubules, is indeed present in the cytoplasmic fraction, while the nuclear protein Creb Binding Protein (CBP) can be found exclusively in the nuclear fraction. These control results show the validity of the fractionation procedure. These results are further explained by the findings of the immunofluorescence experiments.

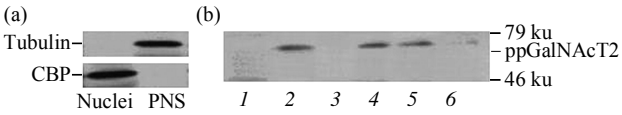

Fig. 5 Subcellular localization of ppGalNAcT2 by biochemical subcellular fractionation

SGC7901 cells were homogenized and fractionated, and 15 mg of protein from the nuclei, post-nuclear supernatant (PNS), 10K and 100K pellet and supernatants were separated by SDS-PAGE. (a) α-Tubulin and Creb binding protein (CBP) are mainly found in the PNS and nuclear fraction. (b) In contrast, anti-T2 Ab recognized a band of approximately 70 ku in PNS, 100K pellets, but not in nucleus or 10K pellets. *1*: Nuclei; *2*: PNS; *3*: 10K Pellet; *4*: 10K Supernatant; *5*: 100K Pellet; *6*: 100K Supernatant.

2.5 Application of anti-T2 antibodies for immunocytochemical localization

To further determine the subcellular localization of ppGalNAcT2 in an independent and complementary approach, SGC7901 cells were probed with the anti-T2 (Figure 6a*A*, b*A*) and viewed with the confocal microscope [22]. The signals obtained when probing the cells with antibody to GS28, a marker for *cis*-Golgi

stacks, and TGN38, a marker for the *trans*-Golgi network (Figure 6b*B*) resulted in a typical Golgi pattern (Figure 6a*B*). For colocalization studies, the pinhole for detecting red fluorescence was set at one Airy unit that corresponded to an imaged depth of ～0.8 μmol/L. The pinhole for detecting green fluorescence was set to image a similar depth. Thus, overlap of the fluorescence constituted colocalization in closely apposed if not identical regions. When the signals were merged, there was significant overlap, suggesting colocalization of the ppGalNAcT2 with the GS28 and TGN38 marker (Figure 6a*C*, b*C*). Data show that not all of the ppGalNAcT2 signals in the juxtanuclear regions overlapped with the Golgi marker, and it was clear that even within the Golgi regions, the ppGalNAcT2 was not uniformly present. Morphometric analyses of several images revealed that ～60% of the ppGalNAcT2 (red) pixels colocalized with the GS28 (green), and ～36% of the GS28 (green) pixels colocalized with the ppGalNAcT2 (red) pixels (Figure 6a*D*). This suggests that ～60% of the ppGalNAcT2 was present in the *cis*-Golgi and that 36% of the *cis*-Golgi contained ppGalNAcT2. Meanwhile, ～34% of the ppGalNAcT2 (red) pixels colocalized with the GS28 (green), whereas ～38% of the TGN38 (green) pixels colocalized with the ppGalNAcT2 (red) pixels (Figure 6b*D*). This suggests that around 34% of the ppGalNAcT2 was in the *trans*-Golgi network and that 38% of the *trans*-Golgi network contained ppGalNAcT2.

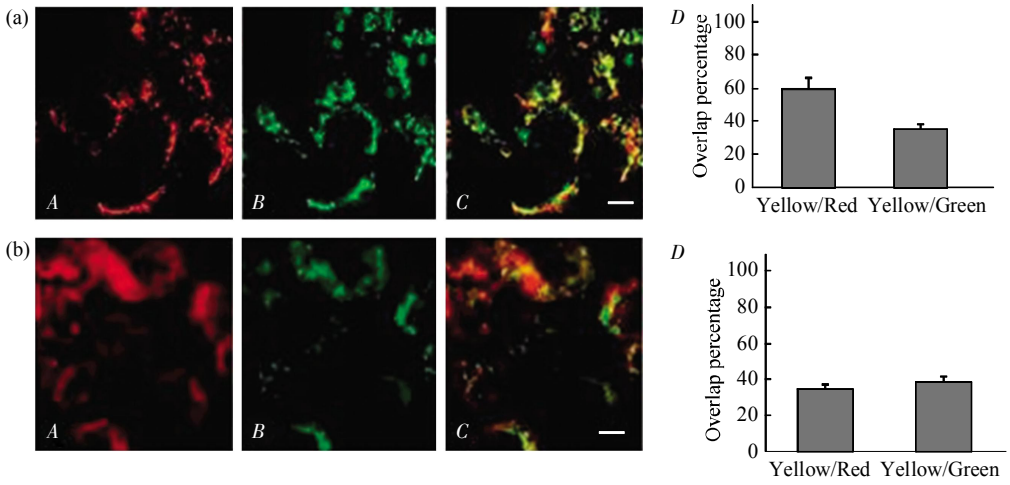

Fig. 6　Localization of ppGalNAcT2 within the *cis*- and *trans*-Golgi apparatus in SGC7901 cells

(a) Cells were probed with rabbit anti-T2 (*A*) and mouse anti-Golgi SNARE (soluble N-ethylmalemide-sensitive factor attachment protein receptor) of 28 ku (GS28) (*B*), followed by anti-rabbit IgG conjugated with Cy3 and anti-mouse IgG conjugated with FITC. Colocalization is represented by yellow when images *A* and *B* are merged (*C*, bar=5 μm). *D*: Percent colocalization of ppGalNAcT2 and GS28 signals. Yellow/Red represents the percent of ppGalNAcT2 signals that colocalize with GS28 signals; yellow/ green represents the percent of GS28 signals that colocalize with ppGalNAcT2 signals. Data represent $\bar{x}\pm s$ from analysis of four different images. (b) Cells were probed simultaneously with rabbit anti-T2 (*A*) and mouse anti-trans-Golgi network (TGN) 38 (*B*), other data analyzed the same way as (a).

3　Discussion

In this report, we describe production and characterization of an antibody to recombinant human UDP-GalNAc : polypeptide α-N-acetylgalactosaminyl-transferase 2 (ppGalNAcT2). The primary structure of ppGalNAcTs is similar to other type Ⅱ Golgi membrane glycosyltransferases containing a catalytic domain but the ppGalNAcTs are unique among glycosyltransferases in possessing a C-terminal, ricin-type lectin domain of ～130 residues containing 3 putative carbohydrate binding sites[18] (Figure 1a*A*). Biochemical analyses suggest that this domain has functions to specific substrates[23～26]. Results of our bioinformatics analysis show the lectin domain of ppGalNAcT2 has lower similarities in homology from those of the other members of ppGalNAcTs (Figure 1b). Considering both immunogenicity and specificity of the expected antibody, we express the GST-T2-lectin fusion protein as our antigen (Figure 1a*D*, Figure 2). To eliminate the crossreaction of the antibodies to GST, we purified the antiserum by two-step affinity chromatography (Figure 3). The results of immunoblot using purified anti-T2 indicate that antibodies to ppGalNAcT2 do not crossreact with GST and can thus be considered monospecific

(Figure 4). By immunoblotting of SGC7901 cell lysates (Figure 4b*3, 4*), our antibodies detected a single band at around 70 ku. Since ppGalNAcT2 has a theoretical molecular mass of about 64.7 ku, and there are reports indicating that many mammalian glycosyltransferases are posttranslationally modified by glycosylation, especially N-glycosylation [27], we hypothesize that the observed difference in the molecular mass may be due to posttranslational modification.

We further examined the intracellular localization of ppGalNAcT2 in cultured cells. As this enzyme initiate O-glycosylation of proteins by addition of GalNAc, its distribution at the ultrastructural level has implication for the topology of O-glycosylation. The findings of subcellular fractionation indicate the association of ppGalNAcT2 with the Golgi apparatus in SGC7901, but not nucleus, plasma membrane or other heavy membrane (Figure 5). Using laser confocal microscopy we have further shown colocalization of ppGalNAcT2 with GS28 and TGN38 (Figure 6), which are accepted markers for the *cis*- and *trans*-Golgi, respectively [28, 29]. Based on morphometric analyses, we estimate that ～60% of the ppGalNAcT2 signal is colocalized with the *cis*-Golgi marker and ～34% with the *trans*-Golgi marker. It is important to note that within a given cell, not all Golgi contain ppGalNAcT2. Only 36% of the *cis*-Golgi marker colocalized with ppGalNAcT2, whereas 38% of the *trans*-Golgi marker colocalized with ppGalNAcT2. This suggests that there may be functional differences between regions of the Golgi complex with regard to glycoprotein assembly. These data support findings reported previously that GalNAc-transferase activity to be localized on the *cis* side of the Golgi apparatus [11], however, it also argues that O-glycans are initiated throughout the Golgi stack.

ppGalNAcTs are mucin-type O-glycosyltransferases. The biosynthetic pathway for the oligosaccharide chains of mucin glycoproteins in normal epithelial cells has been shown to progress from rER→Golgi apparatus→secretory granules [30]. Evidence have become accumulate that variations from the distribution of ppGalNAcTs occur among different cell types and during malignant transformations. Studies by Roth *et al*. [11] indicate that the initial step in porcine and bovine submaxillary gland cells occurs in the *cis* Golgi apparatus. In a detailed study, Rottger [31] have suggested initiation of O-linked glycosylation throughout the Golgi apparatus in HeLa cells. Our observation of ppGalNAcT2 subcellular localization sites in SGC7901 cells indicates that, in stomach cancer cells, mucin-type O-glycosylation may take place in both *cis* and *trans*-Golgi apparatus. Because early acting Golgi glycosyltransferases are located in ER, *cis* and medial compartments of Golgi whereas late acting enzymes are mainly found in the *trans*-Golgi cisternae or the *trans* Golgi network (TGN)[2], ppGalNAcT2 may not only initiate O-linked glycosylation but also has the role in late glycosylation. Nevertheless, further studies still need to be conducted to find out the site in normal cells which we are in progress. Moreover, our results cannot completely exclude GalNAc addition at earlier stages in the secretory pathway since yet unknown or uncharacterized members of the ppGalNAcTs family may be present in the rER or the ERGIC.

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多肽:N-乙酰氨基半乳糖转移酶 2 在 SGC7901 细胞中同时定位于高尔基体顺面囊和反面囊 *

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摘要 多肽：N- 乙酰氨基半乳糖转移酶(ppGalNAcT)在高尔基体中催化粘蛋白型 O- 糖基化的第一步．首先进行了人 ppGalNAcT2 多克隆抗体的制备和鉴定，进一步通过对分离的亚细胞结构进行蛋白质印迹分析，免疫细胞化学后共聚焦显微镜观察此抗体和两个高尔基体标记 GS28(顺面高尔基体的分子标志)和 TGN38(反面高尔基体的分子标志)来研究 ppGalNAcT2 在 SGC7901 细胞株中的亚细胞定位．结果表明：约有 60%的 ppGalNAcT2 信号和 GS28 共定位，大约 36%的 ppGalNAcT2 信号和 TGN38 共定位．约有 34%的 TGN38 和 ppGalNAcT2 信号重叠，而约 38%的反面高尔基体标志和 ppGalNAcT2 重叠．结论是：在 SGC7901 中，ppGalNAcT2 同时定位于高尔基体顺面囊和反面囊中，实验证实了在高尔基体中进行粘蛋白型 O-糖基化的起始反应．

关键词 ppGalNAcT，高尔基体，O- 糖基化，定位

学科分类号 Q53，R34

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