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Expression of N-Acetylglucosaminyltransferase III Promotes Trophoblast Invasion and Migration in Early Human Placenta

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Abstract

Introduction: Trophoblast migration and invasion at the maternal–fetal interface are crucial events for normal placentation and successful pregnancy. This progress is well controlled by many placenta-specific factors. Inadequate trophoblast invasion results in poor placenta plantation or even complications such as preeclampsia. It has been shown that N-acetylglucosaminyltransferase III (GnT-III) participates in tumor invasion and metastasis as a suppressor; however, the expression of GnT-III and its role in normal pregnancy is unclear. Our objective was to characterize GnT-III expression and function during placental development and identify the underlying mechanisms. **Methods:** The expression of GnT-III in human placental tissue from the first trimester was determined by immunohistochemistry. The HTR8/SVneo cell line was used to investigate the effects of GnT-III on proliferation, apoptosis, migration/invasion, matrix metalloproteinase (MMP) 2/9 activity, and the expression of the tissue inhibitor of metalloproteinase (TIMP) 1/2 using cell 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assays, flow cytometric analysis, transwell migration/invasion assays, gelatin zymography, and Western blot, respectively. Moreover, a placental villous explant model was employed to determine its functions in placentation. **Results:** In the first-trimester placental tissue, GnT-III was localized within the cytotrophoblast, the syncytiotrophoblast and the trophoblast columns of human placental villi, decidual cells, and some extravillous cells in the maternal decidua. GnT-III silencing significantly inhibited HTR8/SVneo cell invasion and migration as well as extravillous explant outgrowth. The application of GnT-III siRNA significantly attenuated MMP2/9 activity and increased TIMP1/2 expression. **Discussion and Conclusion:** GnT-III is expressed in trophoblasts during normal human pregnancy and is involved in regulating trophoblast function.

Keywords

N-acetylglucosaminyltransferase III (GnT-III), placenta, trophoblast, invasion/migration

Introduction

The placenta is a transient organ that forms during pregnancy and contributes to successful pregnancy.[1,2] During human placental development, trophoblast invasion is very important. In the early stage of pregnancy, cytotrophoblast (CTBs) cells acquire an invasive phenotype and differentiate into extravillous trophoblasts (EVTs), which can invade the decidua and a portion of the myometrium or remodel the maternal vasculature. The syncytiotrophoblast (STB) is a terminally differentiated cell layer formed by the fusion of multiple CTBs. The STBs can cover the floating villi and mediate nutrient and gas exchange between the fetus and the mother.[3] During these processes, both the maternal uterine epithelium surface and the outer trophoblast layer of an implanting embryo are heavily glycosylated.[4-6] Inadequate placental development, trophoblast invasion, and vascular remodeling as well as abnormal placental angiogenesis have been reported in pathological pregnancies, such as intrauterine growth restriction, preeclampsia, and other obstetrics complications.[7] Consequently, a better understanding of the mechanisms underlying trophoblast invasion may improve our diagnosis and treatment of gestation-related diseases.

Glycosylation is one of the most abundant posttranslational modification reactions.[8] Changes in glycan expression affect many cellular functions, including cell differentiation, transformation, migration, invasion, and immune responses.[9] N-glycan processing or remodeling reactions are catalyzed by several glycosyltransferases, which are highly diverse N-linked glycans (N-glycans) in mammals.[8,10] Among these glycosyltransferases, the 2 major glycosyltransferases are N-acetylglucosaminyltransferase III (GnT-III) and N-acetylglucosaminyltransferase V (GnT-V). GnT-III catalyzes the formation of a bisecting β1,4-GlcNAc structure. GnT-III is a key glycosyltransferase in the N-glycan biosynthetic pathway

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because the introduction of the bisecting GlcNAc residue suppresses further processing and elongation of the N-glycans catalyzed by GnT-V.[11,12] Therefore, in the context of tumors, both GnT-III and GnT-V generally play opposing roles, where GnT-III acts as a metastasis suppressor and GnT-V is associated with increased malignancy and metastasis.[13,14] As several similarities exist between the behavior of placental cells and that of malignant tumor cells,[15] we confirmed in a previous study that GnT-V is highly expressed in the EVT and endothelial cells of the first-trimester villi. We also found a role for GnT-V in the inhibition of human trophoblast cell invasion and migration during early pregnancy via the direct or indirect regulation of matrix metalloproteinase (MMP) 2/9 activity;[16] however, the role of GnT-III has not been researched in normal human pregnancy.

In this study, we examined GnT-III expression in trophoblast cells and the role of GnT-III in human first-trimester placenta. Our findings might be valuable for future research on some obstetrics complications.

Materials and Methods

Tissue Collection

Human decidua and villous tissues from the first trimester (n = 11, 5-10 weeks of gestation) were obtained from healthy pregnant women who underwent legal abortions of pregnancy for nonmedical reasons in an outpatient operating room. All patients with chronic medical disorders were excluded. Approval was granted by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. Informed consent was obtained from all participants.

After surgery, all the tissue samples were immediately transported to the laboratory. For the immunohistochemistry assay, the samples were washed with ice-cold, phosphate-buffered saline (PBS) 3 times and were fixed in 4% neutral paraformaldehyde; the tissues were then dehydrated in gradient ethanol solutions and embedded in paraffin. For the Western blot experiments, the tissues were washed 3 times with ice-cold PBS and were stored in liquid nitrogen at −80°C until further use. Finally, in regard to the explant cultures, the samples were cut into 1- to 5-mm^3 sections, washed 3 times with ice-cold 0.9% saline, and cultured immediately (see details in explants culture method).

Immunohistochemistry and Immunofluorescence

Immunohistochemistry was performed as previously described.[17] Samples from 11 placentas were used. The sections of villous and decidual tissues from each placenta were divided into 2 groups as follows: 1 for GnT-III staining and 1 for cytokeratin 7 (CK7)/human leukocyte antigen G (HLA-G) staining. The sections (each 5-mm thick) were deparaffinized, rehydrated, and incubated with 3% H_2O_2 for 10 minutes to quench the endogenous peroxidase. After blocking with 20% normal goat serum for 30 minutes, all sections were incubated with primary antibodies to either GnT-III (1:200; Abcam, San Francisco, USA), CK7 (1:200; Abgent, San Diego, USA), or HLA-G (1:400; Abcam, San Francisco, USA) for overnight at 4°C. Nonimmune rabbit immunoglobulin G (IgG) was used as a negative control (NC) instead of the primary antibody. Then, the sections were incubated with horse radish peroxidase (HRP)–conjugated goat anti-rabbit IgG antibody (1:1000; Santa Cruz Biotechnology, Texas, USA) for 1 hour at 37°C after several washes. The immunohistochemical staining was developed with 3,3-diaminobenzidine (DakoCytomation, California) working reagent; the tissue sections were then counterstained with hematoxylin and were observed under a microscope (Olympus IX51, Japan).

To prepare the immunofluorescence samples, the cells were fixed on the cover slip and blocked with 5% goat serum for 1 hour at room temperature. The primary antibody used was the anti-GnT-III antibody (1:100; Abcam, San Francisco, USA). An NC was generated by replacement of the primary antibody with PBS. The secondary antibody was a fluorescein isothiocyanate–conjugated goat anti-rabbit antibody (1:50; Santa Cruz Biotechnology, Texas, USA). The nuclei were stained with propidium iodide (3 mg/mL). Images were acquired with an Olympus BMX-60 microscope equipped with a cooled charge-coupled device sensicamera (Cooke, Auburn Hills, Michigan) and Slidebook software (Intelligent Imaging Innovations, Denver, Colorado).

Cell Culture and RNA Interference

The human EVT cell line HTR8/SVneo has been widely used as a model for studying EVT function in the first trimester. The HTR8/SVneo cells that were used in this study were a kind gift from Dr Charles Graham (Queen's University, Kingston, Ontario, Canada), as previously mentioned.[16,18] The cells were routinely cultured in RPMI-1640 medium (Gibco, Massachusetts, USA) supplemented with 10% fetal bovine serum (FBS) at 37°C in an incubator with 5% CO_2.

For GnT-III RNAi, the cells were transfected with 100-nM GnT-III siRNA (5′-CGTCAACCACGAGTTCGACCT-3′, GenePharma, Shanghai, China) or the NC siRNA (a universal NC; GenePharma) with Lipofectamine™ 2000 (Invitrogen, Carlsbad, California), and the transfection procedures were performed according to the manufacturer's protocol.

Western Blot

The cells from the 2 groups were lysed in radio-immunoprecipitation assay (RIPA) lysis buffer (Sigma, Ohio, USA), and Western blot analyses were conducted as previously described.[19] Primary antibodies against the following proteins were used: GnT-III (1:500; Abcam, San Francisco, USA), β-actin (1:1000; Santa Cruz Biotechnology), and tissue inhibitor of metalloproteinase (TIMP) 1/2 (1:1000; Santa Cruz Biotechnology). The membranes were incubated with the primary antibody at 4°C overnight and were then incubated with a rabbit HRP-conjugated secondary antibody (1:2000; Santa Cruz Biotechnology). Immunoreactive signals were detected by enhanced chemiluminescence reagents and were analyzed by a ChemiDoc image analyzer (Bio-Rad).

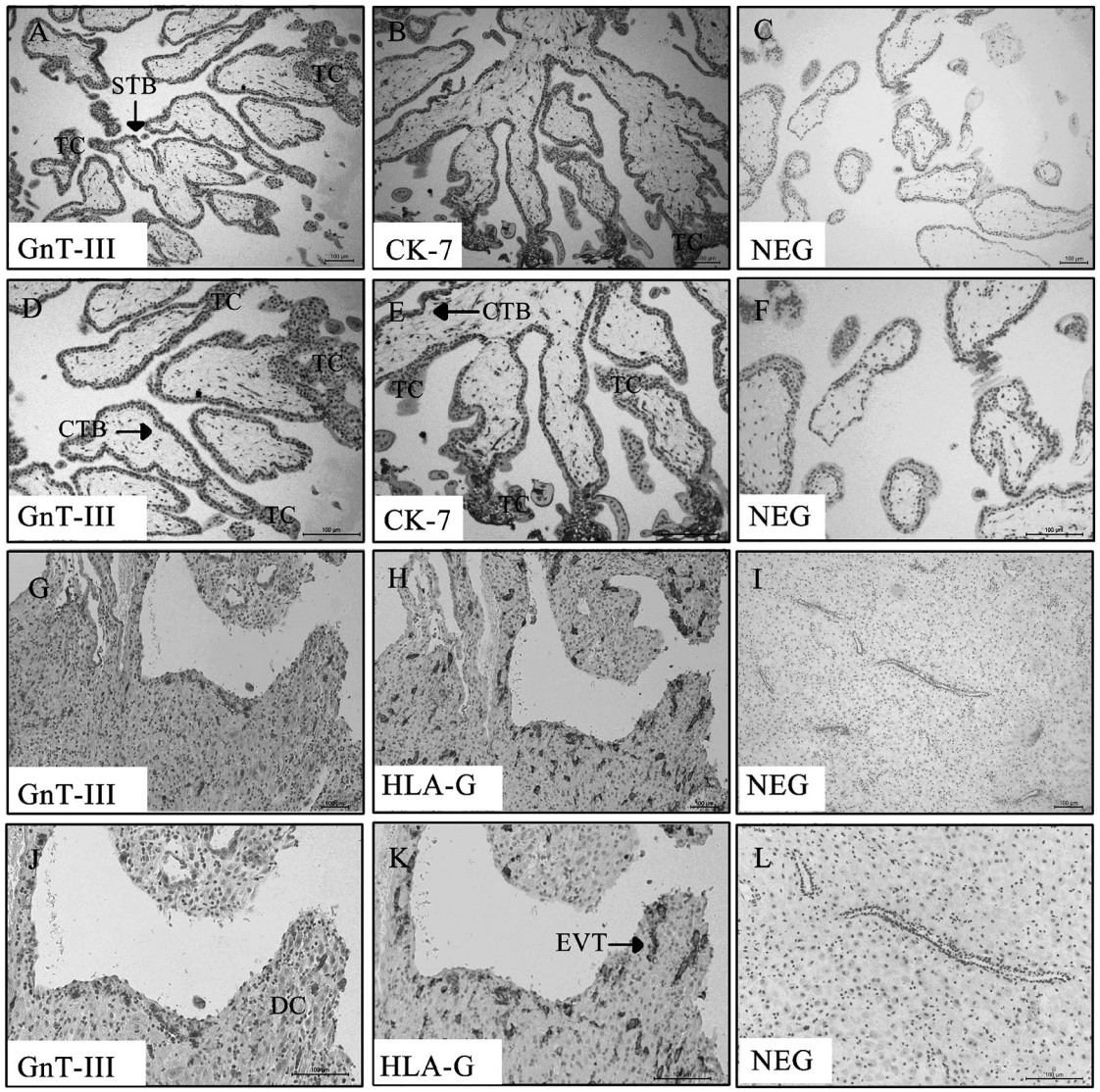

Figure 1. Expression of GnT-III in human placental tissues. A, GnT-III protein was localized within villous cytotrophoblast (CTB) cells, syncytiotrophoblast (STB) cells, and trophoblast columns (TCs) in human placental villi. B, Immunostaining for cytokeratin 7 (CK7), which serves as a marker for CTBs and TCs. C, Negative control (NC) with nonimmune rabbit IgG in villi. D-F, Higher powered images of (A-C). G, GnT-III protein was localized within the decidual cells (DCs) and extravillous trophoblasts (EVTs) in the maternal decidua. H, Immunostaining for human leukocyte antigen G (HLA-G), which serves as a marker for EVTs. I, NC with nonimmune rabbit IgG in the maternal decidua. J-L, Higher powered images of (G-I). Scale bar: 100 μm. GnT-III indicates N-acetylglucosaminyltransferase III.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from the HTR8/SVneo cells with TRIzol reagent (TaKaRa, Japan), and the RNA concentration was measured by ultraviolet spectroscopy (NanoDrop 2000; Thermo, USA). Reverse transcription was performed with a PrimeScript real-time reagent kit with gDNA Eraser (TaKaRa, Japan). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control for gene expression analysis. The sequences of the polymerase chain reaction (PCR) primer pairs for each gene were as follows: GnT-III forward: 5′-TCCGCCACAAGGTGCTCTAT-3′, reverse: 5′-GGATC TCGTCCGCATCGTCA-3′; GAPDH forward: 5′-CTTTGG TATCGTGGAAGGACTC-3′, reverse: 5′-GTAGAGGCAGG GATGATGTTCT-3′. The PCR conditions were as follows: 94°C for 3 minutes, 35 cycles at 94°C for 20 seconds, 58°C for 30 seconds, and extension at 72°C for 30 seconds. All experiments were performed in triplicate.

Cell Invasion and Migration Assay

For the cell invasion assay, transwell inserts (Costar, Cambridge, Massachusetts) were precoated with matrigel (BD Biosciences,

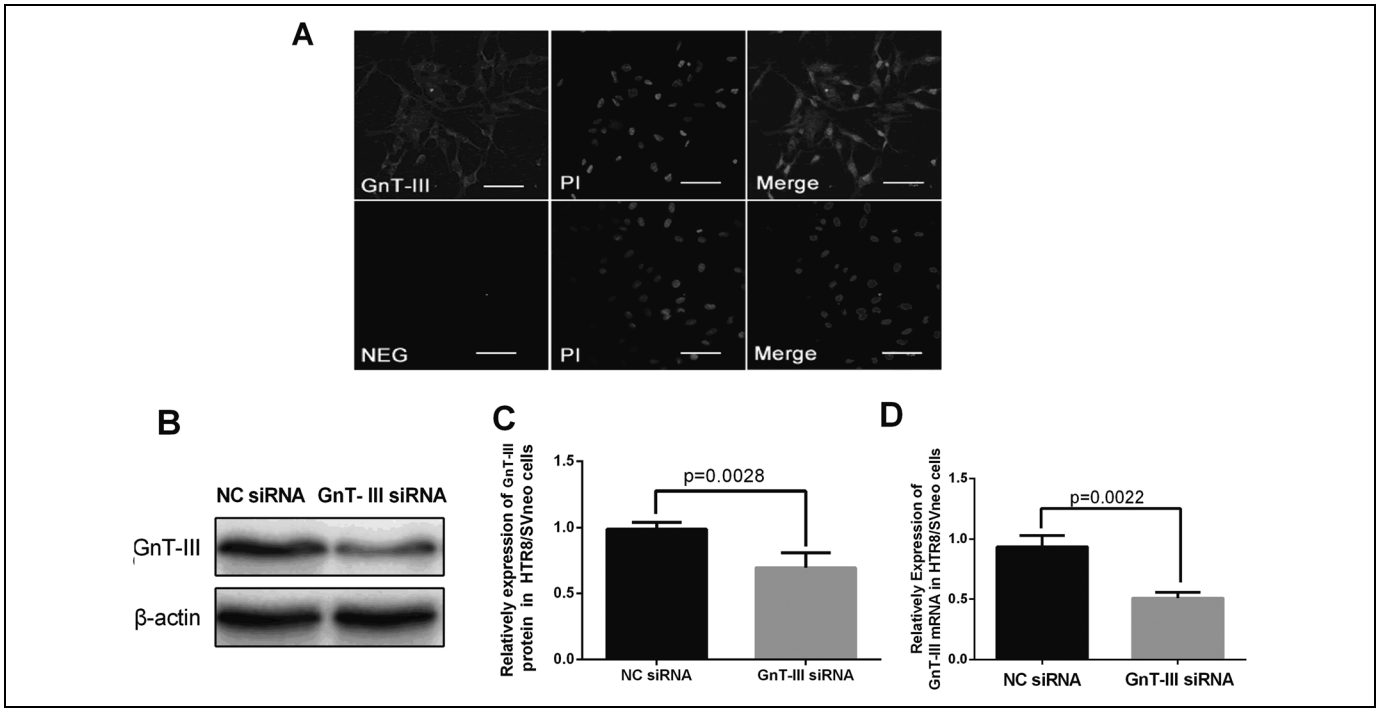

Figure 2. Expression of GnT-III in the HTR8/SVneo cell line and the GnT-III silencing efficiency in HTR8/SVneo cells. A, Immunofluorescence of GnT-III expression in the HTR8/SVneo cell line. Fluorescence signals specific to the GnT-III antibody were visualized as green, and the nuclei were stained with PI (red). NC was generated by replacement of the primary antibody with PBS. Scale bar, 75 mm. B, Representative Western blot image of GnT-III knockdown. C, Statistical bar graphs of the Western blot results in (B). D, Expression of GnT-III mRNA with the indicated treatments. GnT-III indicates N-acetylglucosaminyltransferase III; PI, propidium iodide; NC, negative control; PBS, phosphate-buffered saline.

San Jose, California; 1:9 dilution), as previously described.[16] For the cell migration assay, the transwell inserts were not precoated with matrigel. In all, 1.0×10^5 HTR8/SVneo cells (GnT-III siRNA group or NC siRNA group) in serum-free medium were placed in the upper chamber, whereas 600 mL of medium supplemented with 10% FBS was added to the lower compartment. After incubation for 24 hours, the cells in the upper chamber of the inserts were scrubbed with a cotton swab. Then, the inserts were fixed in methanol and stained with crystal violet. To estimate the number of cells that had invaded or migrated, the cells on the other side of the insert in 5 random fields were counted by light microscopy (Olympus IX51) at a magnification of ×200. The data are shown as the fold change compared with the NC values. Independent experiments were performed in triplicate to reduce intraassay variability.

Cell Proliferation Assay

Cell viability was measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay as previously described.[17] In all, we seeded 5×10^3 HTR8/SVneo cells in each well in a 96-well plate. After a 20-hour culture, 20 mL of MTT reagent (5 mg/mL) was added to the medium and was gently removed 4 hours later. We then added 150 mL of dimethyl sulfoxide to each well. The optical density of each well was assessed at a wavelength of 570 nm (Bio-Rad Model 550, California). The experiment was repeated 3 times in triplicate.

Flow Cytometric Analysis of Apoptosis

To quantify the rate of apoptosis in HTR8/SVneo cells that were pretreated with siRNA, an annexin V- fluorescein isothiocyanate (FITC) and propidium iodide (PI) apoptosis detection kit (Key-Gen Biotech, Nanjing, China) was used as previously described.[20,21] Briefly, HTR8/SVneo cells were grown in a 6-well plate and were incubated for 48 hours. The cells were then washed twice with ice-cold PBS, collected, and stained in the annexin V-FITC and PI binding buffer for 20 minutes. The stained cells were analyzed by a FACS Vantage SE flow cytometer (BD Biosciences). The following 4 different phenotypes were recognized: (a) annexin V^-/PI^- (lower left quadrant, Q3), predicted to be viable cells; (b) annexin V^+/PI^- (lower right quadrant, Q4), usually predicted to be apoptotic cells; (c) annexin V^-/PI^+ (upper left quadrant, Q1), fragments of damaged cells; and (d) annexin V^+/PI^+ (upper right quadrant, Q2), late apoptotic cells. Therefore, the total percentage of apoptotic cells, including those in the early phases of apoptosis and those in the late phases of apoptosis, were represented. The experiment was performed in triplicate.

Villous Explant Cultures

Placental villous tissues (1-5 mm^3) were dissected from the tips of the villi and explanted as previously described.[16] Serum-free Dulbecco's modified Eagle medium/F12 (Gibco,

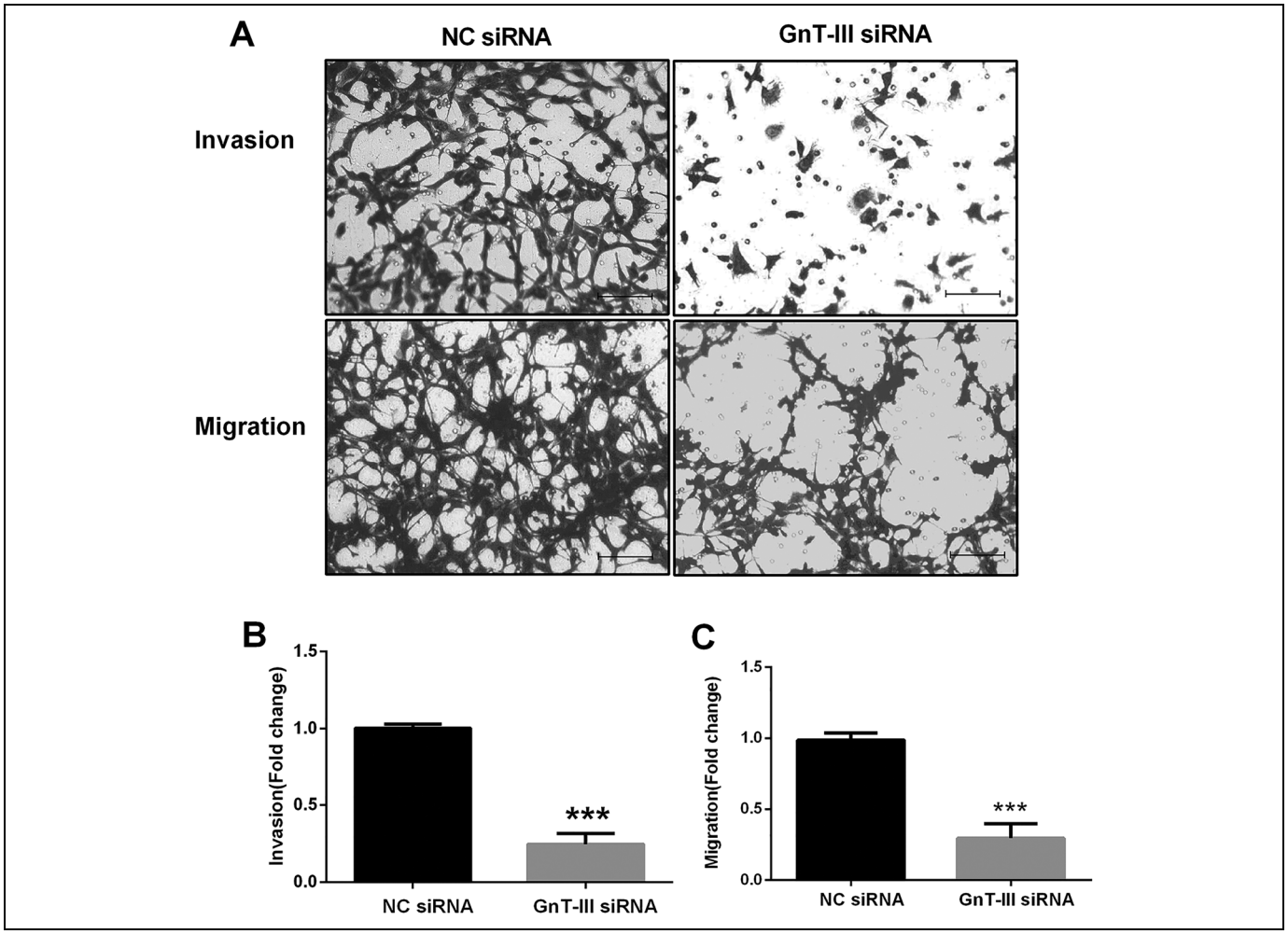

Figure 3. GnT-III siRNA reduced the cell migration and invasion potential in HTR8/SVneo cells. A, Representative images of the migration and invasion assays in HTR8/SVneo cells that were transfected with GnT-III siRNA or NC siRNA. Scale bar, 100 μm. B, Statistical bar graphs showing the summary of 3 independent experiments of the transwell invasion assay. C, Statistical bar graphs showing the summary of 3 independent experiments of the transwell migration assay. GnT-III indicates N-acetylglucosaminyltransferase III; NC, negative control.

Massachusetts, USA) medium with 500 nM siRNA targeting GnT-III or an equal concentration of the NC siRNA was added into the wells. The outgrowth and migration of EVT cells from the distal end of the villous tips were recorded daily for up to 3 days. The migration distance (from the villous tip to the distal edge of the outgrowth sheet) of the EVT cells was measured by manual tracing using ImageJ software (version1.8.0). For each explant, 4 villous tips were randomly chosen, and at least 3 distances were measured for each tip. The explant experiment was performed in quadruplicate and was repeated 5 times.

Gelatin Zymography

Cells (1.0×10^5) were planted in a 24-well chamber and incubated with serum-free medium for 48 hours after siRNA transfection. Zymography was performed as previously reported.[16,17] A total of 10 mL of protein from the conditioned medium of the HTR8/SVneo cells was subjected to electrophoresis. The gels were then washed twice in 2.5% Triton X-100 in 50 mM Tris–HCl for 30 minutes and incubated in calcium assay buffer at 37°C overnight. Finally, the gels were stained with Coomassie Brilliant Blue in 50% methanol and 10% acetic acid and destained in 10% acetic acid. The gels were scanned by densitometry using a CS Analyzer 3.0.

Statistical Analysis

The data are shown as the mean ± standard error of the mean. All statistical analyses were performed with GraphPad Prism (GraphPad Software, San Diego, California). The differences between the 2 groups were analyzed by independent *t* test assuming a Gaussian distribution. A P value <.05 was defined as statistically significant.

Results

GnT-III Was Highly Expressed in Human Placental Trophoblast Cells From the First Trimester

We first examined the expression of GnT-III proteins in different types of trophoblast cells in human first-trimester

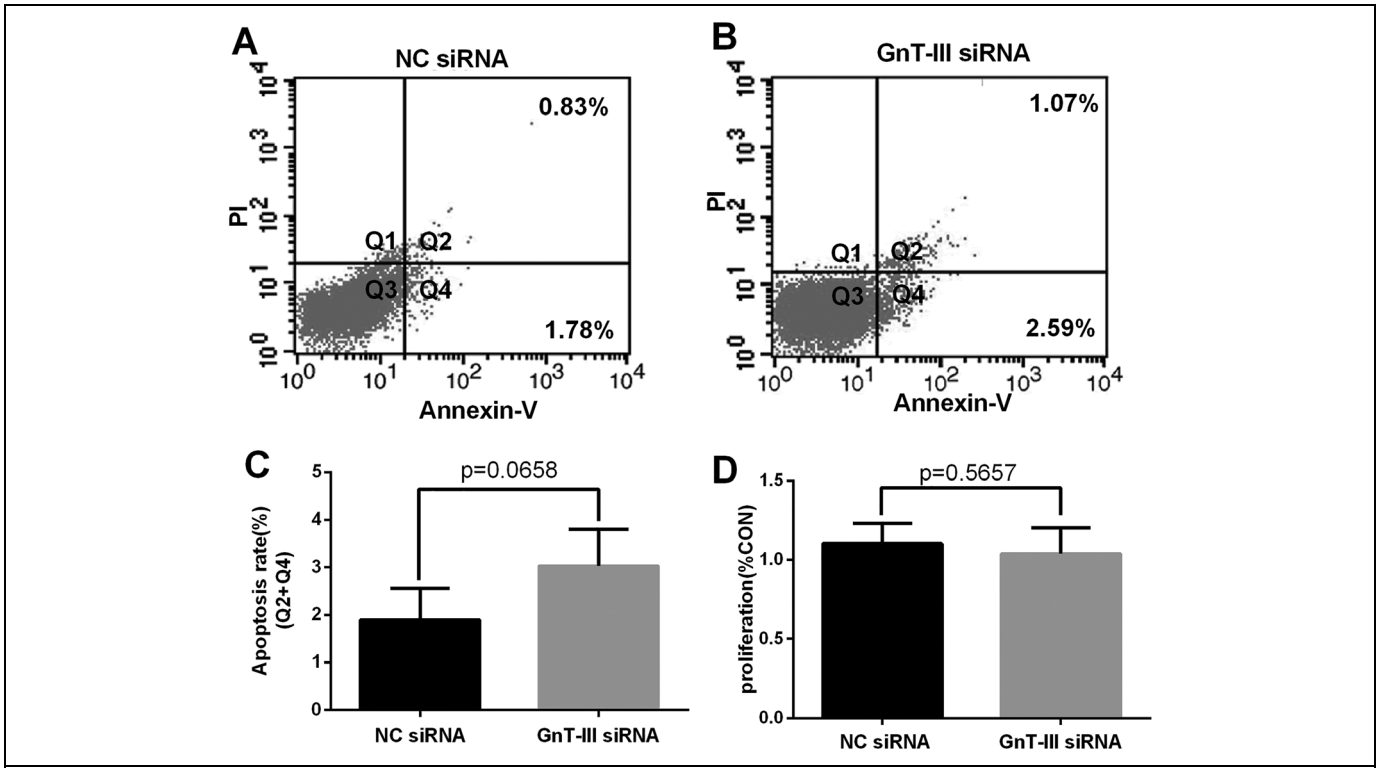

Figure 4. The effect of GnT-III siRNA on apoptosis and proliferation in HTR8/SVneo cells. A-B, Representative images of flow cytometry for the apoptosis rate in the NC siRNA and GnT-III siRNA groups. Annexin V^+/PI^+ (upper right quadrant, Q2); late apoptotic cells. Annexin V^+/PI^- (lower right quadrant, Q4); usually predicted to be apoptotic cells; total percentage of apoptotic cells = Q2 + Q4. B, Ratio of apoptotic cells in a population of HTR8/SVneo cells that was transfected with the indicated siRNA. C, MTT assay of HTR8/SVneo cells transfected with the indicated siRNA in culture. GnT-III indicates N-acetylglucosaminyltransferase III; NC, negative control; PI, propidium iodide; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

placenta. Paraffin sections of villous and decidua tissues from the first-trimester placenta were immunostained with anti-GnT-III. The CK7 immunostaining was used as a marker of villous trophoblasts (Figure 1B and E), and HLA-G served as a marker for EVTs (Figure 1H and K). As shown in Figure 1, the GnT-III protein was intensively and specifically stained in CTBs, trophoblast columns, and STBs in the first-trimester villi (Figure 1A and D) and expressed in the decidual cells and in some EVT cells of the maternal decidua (Figure 1G and J). Immunofluorescence staining confirmed that GnT-III protein was present and expressed in the HTR8/SVneo cell line (Figure 2A).

GnT-III siRNA Significantly Inhibits the Invasion and Migration Capacities of HTR8/SVneo Cells

It is known that trophoblast invasion and migration capacity are vital events in the placenta, and the expression of GnT-III in the trophoblast cell line suggests a regulator role for GnT-III in cell behavior. To determine whether GnT-III siRNA effected trophoblast invasion and migration, matrigel cell invasion and transwell cell migration models were employed. Transfected cells were cultured in parallel to examine the silencing efficiency. The transfection efficiency, as evaluated by the fluorescence signal in FITC siRNA-transfected HTR8/SVneo cells, was almost 90% (data not shown).

Specific siRNA targeting GnT-III significantly decreased GnT-III protein and mRNA in HTR8/SVneo cells (Figure 2B-D, $P < .01$). Compared with the NC siRNA-transfected HTR8/SVneo cells, GnT-III siRNA significantly decreased the percentage of cells that invaded (Figure 3A and B, $^{***}P < .0001$) or migrated (Figure 3A and C, $^{***}P < .0001$) to the other side of the filters.

To rule out the possibility that the decrease in cell numbers that had migrated and invaded was due to the effects of GnT-III on cell proliferation and/or viability, we examined the proliferation and apoptosis of these cells after siRNA transfection. The apoptosis rate and proliferation of HTR8/SVneo cells were not significantly changed with GnT-III siRNA treatment based on flow cytometric analysis and MTT assay (Figure 4, $P > .05$).

Knockdown of GnT-III Inhibits the Migration Ability of EVTs in Extravillous Explants

To further confirm the role of GnT-III in trophoblast invasion and migration in vivo, explants were freshly obtained from 1 placenta and separated into 2 groups. One group was treated with NC siRNA and the other group with GnT-III siRNA. After

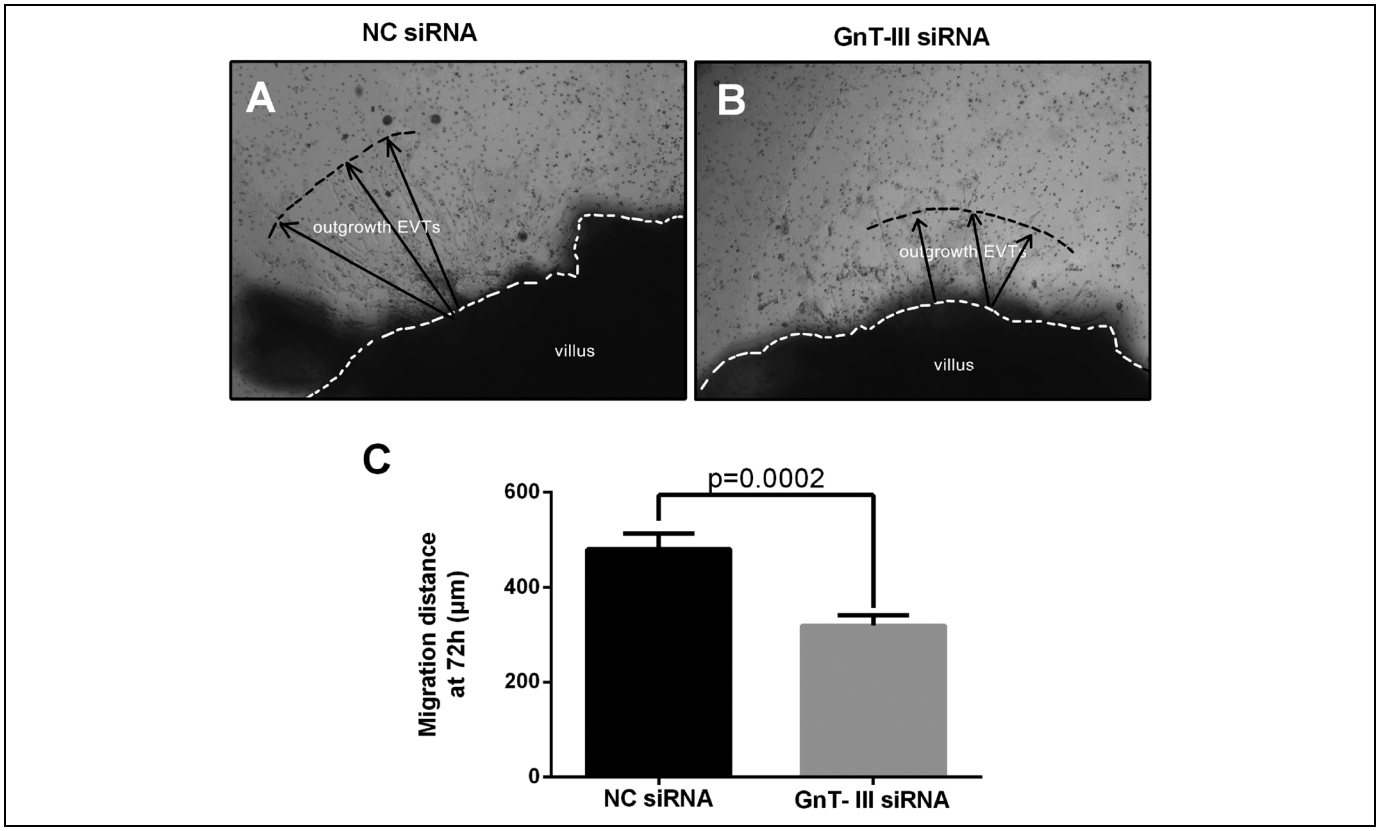

Figure 5. GnT-III siRNA attenuated trophoblast outgrowth and migration in placental villous explant cultures. A-B, Representative image of placental villous explants after a 72-hour culture in vitro (magnification ×40). The outgrowth distance (indicated by the black arrows) of the explants under different treatments. Migratory front and villous tips are displayed by a white dotted line; the length of the cellular displacement is shown by arrows. B, Graphical representation of trophoblast cell migration distance in the placental villous explants. GnT-III indicates N-acetylglucosaminyltransferase III.

a 72-hour culture in vitro, GnT-III siRNA- (319.3 ± 11.03 mm) treated explants displayed a significant reduction in the outgrowth and distance of migration compared with the NC siRNA group (479.5 ± 16.65 mm; Figure 5, $P < .001$).

Effects of GnT-III Knockdown on Gelatinase Activity

The invasion of trophoblasts and cancer cells is a multistep process involving attachment to a basement membrane or extracellular matrix (ECM) components, followed by degradation and subsequent migration through the degraded components. Type IV collagenases, such as MMP2 and MMP9, and the specific inhibitors of TIMP2 and TIMP1 are thought to be the principal mediators of trophoblast and cancer cell invasion. Hence, we examined MMP2/9 and TIMP2/1, respectively, by gelatin zymography and Western blot. GnT-III knockdown significantly decreased MMP9 and MMP2 activity in the supernatants of HTR8/SVneo cells compared with the NC siRNA (Figure 6A and B, $P < .05$), while it had a positive relationship with the expression levels of TIMP2 and TIMP1 (Figure 6A and C, $P < .05$).

Discussion

The glycosylation of glycoprotein plays a key role in a variety of specific biological interactions.[22] In particular, the branching of N-linked oligosaccharides regulates the metastatic potential of cancer cells.[8] GnT-III is one of the glycosyltransferases involved in N-glycan biosynthesis. Although many studies have confirmed the relevance between GnT-III and cancers,[13,23,24] GnT-III expression and its role in trophoblast cells are still a puzzle.

In the present study, by use of immunohistochemistry, we revealed that GnT-III was expressed during normal human pregnancy. In first-trimester villous, GnT-III was localized in CTB cells, STB cells, and the trophoblast columns. In the maternal decidua, GnT-III was expressed in the decidual cells and in some EVT cells. This finding suggests that GnT-III has a role in regulating trophoblast function during placentation. The physiological balance of trophoblast invasion is regulated by a cross talk between trophoblasts and decidual cells in a paracrine or autocrine manner. Therefore, the expression of GnT-III in decidual cells at the maternal–fetal interface is important in controlling the overinvasion of trophoblast cells.[3] We further showed that the silence of GnT-III decreased the invasion and migration potential of the trophoblast cell line HTR8/SVneo but did not affect cell apoptosis and proliferation. Additionally, the knockdown of GnT-V inhibited the outgrowth and migration capacities of first-trimester human placental villi in an in vitro explant culture. An advantage of the villous explant

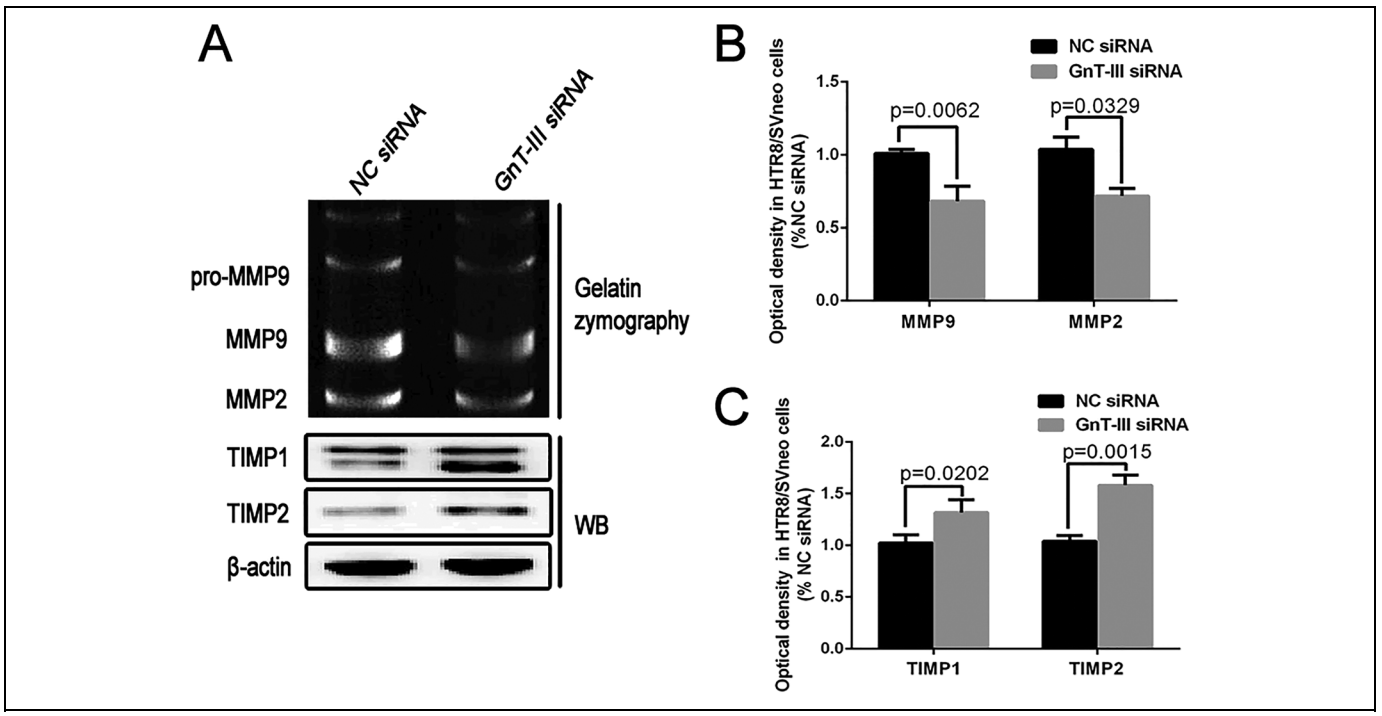

Figure 6. GnT-III knockdown decreased the activity of MMP2/9 and increased the expression of TIMP1/2. A, Representative image of gelatin zymography assay in HTR8/SVneo cells. B, Statistical analysis of the gelatin zymographic results in (A). C, Statistical analysis of the Western blot results in (A). GnT-III indicates N-acetylglucosaminyltransferase III; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

model, compared with primary trophoblast cells, is that this model reflects the complicated relationship among the different types of cells and maintains the integrity of the tissue structure.[25] Finally, the downregulation of MMP2/9 and the upregulation of TIMP1/2 were confirmed in GnT-III siRNA-transfected cells.

Our results are inconsistent with previous studies in oncology, which have noted high expression of GnT-III associated with decreased malignancy and metastasis of cancer cells. Further, our previous study on GnT-V also confirmed that GnT-V played a crucial role in the inhibition of trophoblast migration and invasion.[16] Such a conflict may have resulted from the different types of cells or cell lines used in the experiments.[13,23,24] In oncology, studies have investigated the role of GnT-III in the processes of migration and invasion using cancer cell lines; however, trophoblast cells and cancer cells share several similarities in cell behavior, but placental cells are not malignant. The invasion capability of trophoblast cells is self-limited and is well regulated by placenta-specific genes.[26,27] This regulation ensures that the invasion of EVTs only occurs during early pregnancy and that it is restricted to the proximal third of the myometrium.[27]

In human placenta, EVTs invasion involves degradation of the ECM. The migratory trophoblasts express MMPs, urokinase plasminogen activator, and cathepsins, whereas decidua produces TIMPs and plasminogen activator inhibitor to restrict trophoblast invasion.[28,29] In this study, GnT-III siRNA was shown to be able to decrease the gelatinolytic activity of MMP2/9 in HTR8/SVneo cells and increase TIMP1/2 expression in GnT-III knockdown cells. The precise mechanism through which pathway GnT-III can regulate the activity of MMPs, or whether these MMPs are direct targets for GnT-III, requires further elucidation.

In adhesion molecules, integrin and E-cadherin are major carriers of N-glycans. The changes of biological functions of both molecules by N-glycosylation are associated with a carcinogenic process and tumorigenesis.[8,30] Integrin is a regulator of the interaction between fibroblasts and ECM components, including certain collagens and laminin. Numerous studies have reported that $\alpha 3\beta 1$ integrin (laminin receptor) is a GnT-III target molecule and changes the biological functions in cancers by N-glycosylation catalyzed by GnT-III.[13,31] Thus, a future study needs to confirm the relationship between invasion and glycosylation on $\alpha 3\beta 1$ integrin.

Whether the knockdown of GnT-III can promote trophoblast function and decrease the secretion of antiangiogenesis factors in conditions of placental insufficiency, such as preeclampsia or intrauterine growth restriction, remains to be determined. We have already determined that GnT-III expression is decreased in placentas from pregnancies that are complicated by preeclampsia compared with placentas from normal pregnancies, and future studies should clarify the underlying molecular mechanism between GnT-III expression and preeclampsia.

In summary, our results show that GnT-III is expressed in trophoblast cells and EVTs in the first trimester. We have provided the first evidence of a functional role for GnT-III in trophoblast migration and invasion. Therefore, GnT-III may be an important regulator of placentation.

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Declaration of Conflicting Interests

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