Alpha 2,6-Sialyltransferase I Expression in the Placenta of Patients with Preeclampsia

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Background: The expression of sialyl-glycoconjugates changes during development, differentiation and oncogenic transformation, tumor invasion and metastasis. Similarly, in the early stage of pregnancy, trophoblast cells have to undergo adhesion, invasion, and proliferation to develop a healthy placenta; the cytobiologic behavior is similar to tumor growth and invasion. Inadequate trophoblast invasion to the spiral artery in the 2nd trimester of pregnancy was believed to be correlated with pregnancy complications, including preeclampsia.

Methods: Alterations in α2,6-sialyltransferase I (ST6Gal I) mRNA in the placental tissues of women with preeclampsia ($n = 20$) and without preeclampsia ($n = 20$ used as a control) were examined by semiquantitative reverse transcription-polymerase chain reaction and real-time quantitative reverse transcription-polymerase chain reaction. The transcription regulators of ST6Gal I including a "constitutive" promoter (Y + Z form), "hepatic" promoter (H form), and lymphoblastic promoter (X form) were investigated. The enzyme activity of ST6Gal I was also examined.

Results: Both mRNA expression and enzyme activity of ST6Gal I did not show a significant difference in the placental tissues of the women of both groups. The transcription regulators of ST6Gal I, including the Y + Z form and the H form, also failed to show any difference. The X form, seldom detected in the study, was excluded from analysis.

Conclusion: Our results suggested that ST6Gal I was not involved in the pathogenesis of the preeclampsia. [*J Chin Med Assoc* 2007;70(4):152–158]

Key Words: α2,6-sialyltransferase I, placenta, preeclampsia, pregnancy

Introduction

Sialic acids (SAs), including a number of their derivatives, are ubiquitous at the terminal positions of the oligosaccharides of glycoproteins.[1,2] Due to their acidic nature, they impart a net negative charge to the cell surface and are important in cell–cell or cell–matrix interaction.[3,4] The transfer of SA from cystidine-5-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) to the terminal position of the carbohydrate group of glycoproteins and glycolipids is catalyzed by a family of sialyltransferases (STs).[5] Many studies have demonstrated that altered sialylation, which occurs during certain pathologic processes, such as oncogenic transformation, tumor metastases, and invasion, is associated with enhanced ST activity.[6–15] Many glycoproteins, glycolipids and oligosaccharides contain SA, which may occur in a variety of structures. A separate ST is responsible for the attachment of the SA residue in each of these structures. STs are classified into 4 major groups based on the types of glycosidic linkage: α2,3Gal (ST3 Gal: ST3β-galactoside α2,3-sialyltransferase), α2,6Gal (ST6Gal: ST6β-galactosamide α2,6-sialyltransferase), α2,6GalNAc (ST6GalNAc: [α-*N*-acetyl-neuraminyl-2,3-β-galactosyl-1,3]-*N*-acetylgalactosaminide α-2,6-sialyltransferase), and α2,8Gal STs (ST8Sia: ST8 α-*N*-acetyl-neuraminide α2,8-sialyltransferase).[16] SA and their derivatives are ubiquitous at the terminal positions of oligosaccharides of glycoproteins and glycolipids in tissues. They play important roles in a large variety of biological processes such as cell–cell communication, cell–matrix interaction, and the maintenance of serum

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glycoproteins in circulation. The expression of sialylglycoconjugates changes during development, differentiation and oncogenic transformation, tumor invasion and metastasis. Similarly, in the early stage of pregnancy, trophoblast cells have to undergo adhesion, invasion and proliferation to develop a healthy placenta; the cytobiologic behavior is similar to tumor growth and invasion.

Preeclampsia is a common disease occurring in pregnant women, with an incidence of 2–5%. The diagnosis of preeclampsia includes hypertension, proteinuria, and general edema. Its correlation with maternal mortality is followed by postpartum hemorrhage only. So far, the etiology of preeclampsia is not clear, but the possibility of it being the result of failed penetration or communication between extravillous cytotrophoblastic cells and the spiral artery of the uterus in the second trimester (14–16 weeks of gestational age) has been suggested.[17] This inadequate trophoblast invasion results in a defective blood exchange between the uterus and placenta, and further contributes to the compensatory elevation of maternal blood pressure.[18,19] According to literature reviews, activities of either α2,6 or α2,3 STs have been found in placental tissue. [20,21] A study of the expression of these enzymes in the placental tissue of preeclampsia patients has never been reported. Based on observations of tumor behavior, we highly suspected that the expression of these enzymes would be significantly decreased. Therefore, we used semiquantitative reverse transcription-polymerase chain reaction (SQ-RT-PCR) and real-time quantitative reverse transcription-polymerase chain reaction (RTQ-PCR) to compare the expression of α2,6-sialyltransferases I (ST6Gal I), which is the most important enzyme during disease transformation such as oncogenic transformation, in placental tissue from patients with and without preeclampsia. At the same time, the enzyme activity of ST6Gal I was also examined.

Methods

Tissue collection

This prospective study involved 20 patients with preeclampsia, who underwent cesarean section for obstetric reasons, and 20 healthy and matched pregnant women without preeclampsia, but who were also undergoing cesarean section for obstetric reasons between January and December 2003. Normal pregnancy was defined as a pregnancy in which the mother had normal blood pressure (≤ 140/90 mmHg), no proteinuria, and an absence of medical and obstetric complications. Preeclampsia was defined as maternal systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on 2 occasions, separated by 6 hours and proteinuria > 300 mg in a 24-hour period. To make this study even more uniform and consistent, the tissue was collected from women with mild preeclampsia only. Cases of severe preeclampsia were excluded from this study. Preeclampsia was considered severe if 1 or more of the following criteria were present: maternal blood pressure ≥ 160/110 mmHg on 2 separate readings at least 6 hours apart; proteinuria > 3+ or > 5 g/24 hours after 20 weeks of gestation; oliguria of less than 500 mL in 24 hours; cerebral or visual disturbances; and pulmonary edema or cyanosis. Patient consent for the collection of placental tissues was obtained from all women, according to the guidelines of the Human Ethics Committee in our institute. The placental tissues were obtained during the cesarean section procedure. Fetal membranes and maternal decidua were removed, and villous tissue was cut into approximately 1-inch cubes and washed extensively with saline in order to remove the blood. The cubes were immediately frozen at –70°C for subsequent enzyme activity and SQ-RT-PCR and RTQ-PCR analyses.

SQ-RT-PCR

Tissue was snap-frozen in liquid nitrogen until RNA extraction. Forty milligrams of tissue were powdered in liquid nitrogen. The method of RNA extraction and SQ-RT-PCR has been described in detail.[13,14,22] Briefly, RNA was extracted using a StrataPrep total RNA Miniprep Kit (Stratagene, La Jolla, CA, USA). Reverse transcription into cDNA was achieved by using the SuperScript™ Preamplification System for First Strand cDNA Synthesis (Life Technologies, Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol, with oligo(deoxythymidine) as the initiation primer in a final reaction volume of 20 μL. The synthesized cDNA in 1 μL was subjected to PCR amplification using 4 pairs of ST-specific primers, respectively, and the β-actin-specific primers. Meanwhile, the extracted RNA from the individual sample was subjected to the following PCR reaction as a negative control.

The condition used for the PCR was set up based on the titration experiment conducted, which is summarized in Table 1. The PCR mixture consisted of 0.1 U Super-Therm DNA polymerase (JMR Holdings, St. Louis, MO, USA), 1 × PCR buffer (JMR Holdings), 1.5 mM $MgCl_2$, 320 nM dNTPs, 0.8–1.6 μL of 10 μM ST primers, 0.8–1.6 μL of 10 μM β-actin primers, 1 μL of cDNA and extra-distilled water added to 50 μL (Table 2). All PCR experiments were conducted in the GeneAmp PCR system 2400 (Applied Biosystems, Singapore).

Table 1. Condition for semiquantitative reverse transcription-polymerase chain reaction (SQ-RT-PCR)

Program	6Gal-I		6Gal-I Y+Z type		6Gal-I H type		6Gal-I X type	
1	94°C	1 min	94°C	2 min	94°C	2 min	94°C	2 min
2	65°C	50 sec	94°C	1 min	94°C	1 min	94°C	1 min
3	72°C	2 min 40 sec	59°C	30 sec	49°C	30 sec	63°C	30 sec
4	1–3 repeated 5 times		72°C	50 sec	72°C	50 sec	72°C	50 sec
5	94°C	50 sec						
6	67°C	50 sec						
7	72°C	2 min 40 sec	2–4 repeated 35 times		2–4 repeated 35 times		2–4 repeated 35 times	
8	5–7 repeated 25 times							
9	72°C	10 min	72°C	10 min	72°C	10 min	72°C	10 min
10	4°C	10 min	4°C	10 min	4°C	10 min	4°C	10 min

Table 2. Primers used for target genes

Target cDNA	Acceptor specificity	Primers	PCR product size (bp)
ST6Gal I	β-galactoside α2,6-sialyltransferase	6gl(f)-5′-TAT CGT AAG CTG CAC CCC AAT C-3′ (GenBank accession no. X17247 1281-1302) 6gl(r)-5′-TTA GCA GTG AAT GGT CCG GAAG-3′ (GenBank accession no. X17247 1652-1631)	372
ST6Gal I Y+Z form	β-galactoside α2,6-sialyltransferase	Z01(f)-5′-AGT CCA GGG AGA AGT GGT GA-3′ (GenBank accession no. X17247 148-167) Z01(r)-5′-CCA CAC ACA GAT GAC TGC AA-3′ (GenBank accession no. X17247 509-490)	362
ST6Gal I H form	β-galactoside α2,6-sialyltransferase	H01(f)-5′-TGT CTC TTA TTT TTT GCC TT-3′ (GenBank accession no. Z35760, nucleotides 422-441) H01(r)-5′-GGT GTG AAT CATAAT GAA GA-3′ (GenBank accession no. X17247, nucleotides 443-424)	218
ST6Gal I X form	β-galactoside α2,6-sialyltransferase	X01(f)-5′-CTT CTC CCA TAC CTT GCT CTA CA-3′ (GenBank accession no. NM_003032 55-77) X01(r)-5′-GAA GAT GTG TTC AGG GAA GTC AC-3′ (GenBank accession no. X17247 428-406)	253
β-actin		β-actin(f)-5′-GGC ATC GTG ATG GAC TCC G-3′ (GenBank accession no. BC004251 448-466) β-actin(r)-5′-GCT GGA AGG TGG ACA GCG-3′ (GenBank accession no. BC004251 1042-1060)	613

Reaction products obtained were electrophoresed in 2% agarose containing ethidium bromide. Density measurements were made using an Alpha-Imager 2000. The density of each ST band was compared with that of β-actin, which was co-amplified within the same tube, and the ratio (density units of ST band/density units of β-actin) was calculated. The detection limit was 1 ng of double-stranded DNA. The linear portion of the assay ranged up to 25 ng. The ST mRNA expression of each sample was determined in at least 3 independent experiments. Using β-actin as an internal standard, the deviation between triplicate measurements was on average 21%.

RTQ-PCR for ST6Gal I, ST6Gal I Y+Z form, ST6Gal I H form, and ST6Gal I X form

The detailed procedure has been reported previously.[22,23] Briefly, kinetic PCR was performed on a LightCycler (RocheDiagnostics, Mannheim, Germany) using SYBR green I as a double-strand DNA-specific binding dye and continuous fluorescence monitoring.[24–27] The primers for quantitative RT-PCR of ST6Gal I, Y+Z type transcript, H transcript, and X transcript were the same as those used for SQ-RT-PCR. The expression of mitochondrial ATP synthase 6 (mATPsy6)-housekeeping genes was used in the PCR reaction as an internal control. The mATPsy6 coding

region was amplified using the forward primer mATPsy6-f 5′-CAGTGATTATAGGCTTTCGCTC-TAA-3′ (GenBank accession no. AF368271, nucleotide 335-359, Tm 56.4°C) and the reverse primer mATPsy6-r 5′-GGCCAGGGCTATTGGTTGAA-3′ (GenBank accession no. AF 368271, nucleotide 471-452, Tm 60.5°C). Amplification was carried out in a total volume of 20 μL containing 0.5 μM of each primer, 4 mM $MgCl_2$, 2 μL LightCycler-FastStart DNA Master SYBR green 1, and 2 μL of 1:50 diluted cDNA prepared as described above.

In all experiments, a negative control reaction was performed by replacing the cDNA template with sterile water, and positive controls were performed with the CC7T cell line, a cell line that expresses ST6Gal I.

Sialyltransferase assay

The detailed procedure has been reported previously.[14,28] Asialo-fetuin (acceptor for ST6Gal I) was from Sigma (St. Louis, MO, USA). CMP-[^{14}C]NeuAc (100 nCi/μL) came from Amersham (Amersham Pharmacia Biotech, UK). The enzyme assay method was modified from Kurosawa's original design[29] and has been described previously.[14,28] To obtain the quantitative data, experiments were reexamined under the linear condition with time and enzyme concentration (enzyme activity unit, nanomol sialic acid/mg protein/hour).

Statistical analysis

Statistical analysis of data was done using the statistical software SPSS, version 10.0 (SPSS Inc., Chicago, IL, USA). The Mann-Whitney test was used to test differences between the expression of a given ST and its regulator in the placental tissues of patients with and without preeclampsia. Probability values below 5% were considered statistically significant.

Results

No different ST6Gal I mRNA expression in placental tissues between normal controls and preeclampsia patients

Expression of ST6Gal I and its promoters, such as H form, Y + Z form, and X form mRNAs in the placental tissues of pregnant women with preeclampsia (n = 20) and those without preeclampsia (n = 20) were examined by RT-PCR with co-amplification of β-actin. The RNA quality was assessed by running it on an agarose gel, while viability of the RNA in each tissue sample was confirmed by amplification of complementary DNA for GAPDH. The standard deviation between

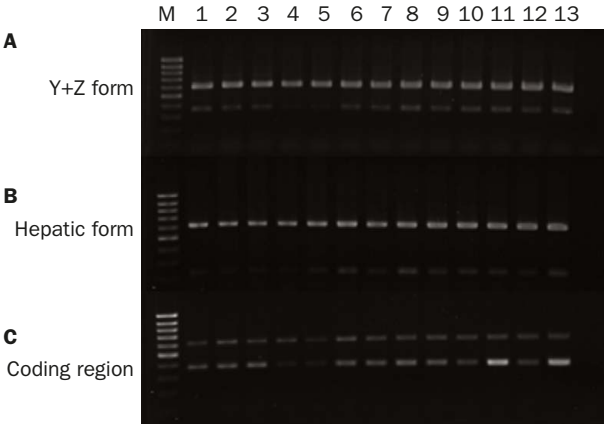

Figure 1. Specificity of RT-PCR products (A = Y + Z promoter; B = H promoter; C = α2,6-sialyltransferase I) in placental tissues of those with and without preeclampsia were documented with high-resolution gel electrophoresis (1.5% agarose gel) and resulted in a single product with the desired length (362 bp for Y + Z form, 218 bp for H form, 372 bp for ST6Gal I, 613 bp for β-actin). Lanes 1–5: normal control placental tissue. Lanes 6–12: preeclampsia placental tissue. Lane 13: CC7T cell line.

triplicate measurements was 21%. In Figure 1, examples of SQ-RT-PCR results of placental tissue samples from women with or without preeclampsia can be seen. ST6Gal I mRNA expression failed to show significant difference between preeclampsia placental tissue and normal control placental tissue (1.09 ± 0.39 *vs.* 1.04 ± 0.42, p = 0.99). To further dissect the controlled promoter for ST6Gal I expression, we studied the H form, the Y + Z form, and the X form. The X form was rarely detected in the placental tissues of preeclampsia or normal control women; therefore, we did not study its expression further. Consistent with the lack of difference of ST6Gal I expression in the placental tissues between normal control women and preeclampsia women, expression of either the H form or the Y + Z form failed to show a significant difference in the studied cases (H form, p = 0.66; Y + Z form, p = 1). Figure 2 summarizes the data in this study. RTQ-PCR further confirmed the above findings, which showed no significant difference between the 2 groups (data not shown).

Low ST6Gal I enzyme activity of placental tissues from normal controls and preeclampsia patients

Consistent with the results of studying the mRNA expression of ST6Gal I from the placental tissue of normal controls and preeclampsia patients, no difference in ST6Gal I enzyme activity was noted between the placental tissue of the normal controls and that of the preeclampsia patients, due to the very low enzyme

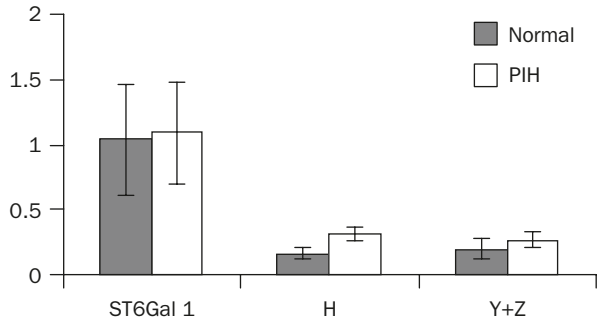

Figure 2. mRNA expression of the α2,6-sialyltransferase I, the H form promoter, and the Y+Z form promoter in placental tissues of those with and without preeclampsia. Data are presented as mean±standard deviation. PIH=pregnancy-induced hypertension (preeclampsia in this study).

activity of the ST6Gal I enzyme in the placental tissues from both groups (data not shown).

Discussion

The current structure of antenatal care has been developed around detecting preeclampsia. The detection of hypertension and proteinuria, the defining signs of this syndrome, is the key aim of frequent surveillance in pregnancy. Preeclampsia occurs in about 3% of pregnant women and results in around 100,000 maternal deaths per year worldwide.[30] The fetus is also affected, directly through placental insufficiency and indirectly through iatrogenic delivery; this accounts for 25% of all infants with a very low birth weight (<1,500 g).[30] Many biochemical substances, principally of placental and endothelial origin, increase in preeclampsia. However, their predictive value in detecting susceptible women before clinical presentation is poor.[30] Therefore, since altered sialylation plays an important role during development, differentiation and oncogenic transformation, tumor invasion and metastasis,[31,32] and of most importance, possibly in the early stage of pregnancy, trophoblast cells have to undergo adhesion, invasion and proliferation to develop a healthy placenta, the cytobiologic behavior is similar to tumor growth and invasion, so it is rational to expect changes in sialylation in the placental tissues of women with preeclampsia. In our previous studies,[13,14,22,23] we found that changes in α2,6 sialylation occurred frequently in oncogenic transformation, and were correlated with the invasive behavior of tumor. In addition, elevation of ST6Gal activity has been reported in human choriocarcinoma (the α2,6 ST activity in JEG-3 and BeWo, 2 choriocarcinoma cancer cell lines, increased to levels several times higher than those in placenta).[33] Competitive PCR analysis showed that the expression levels of mRNA encoding α2,6 ST increased significantly as a result of tumorigenesis,[33] and the inadequate invasion of cytotrophoblasts into the spiral vessels of the uterus was suspected to contribute to the pathogenesis of preeclampsia over the long term.[18,19] The decreased expression of highly invasive molecules such as ST6Gal I could be expected in the placental tissues of women with preeclampsia. However, the enzyme activity of ST6Gal I was very low in the placental tissues from both the normal controls and patients with preeclampsia. The ST6Gal I enzyme activity in the placental tissues of women with and without preeclampsia was not statistically different. Consistent with enzyme activity, ST6Gal I mRNA expression in placental tissue from women with and without preeclampsia was also not statistically different. To further dissect the reality of ST6Gal I expression, we evaluated their control region-regulator parts, including the H form, the Y+Z form, and the X form. Dall'Olio[34] has reviewed the biosynthesis and functional role of ST6Gal in detail. Cell type-specific mRNA isoforms generated by cell type-specific promoters have been identified in human ST6Gal I.[35] A short mRNA form (Form 1) has been isolated from the liver.[36–38] A large transcript (Form 3), containing 2 5′-untranslated exons (exon Y+Z), has been isolated from several human cell types.[36,38–40] A distinct transcript (Form 2) containing exon X, but not exon Y+Z, has been isolated from human B cell lymphoblastoma cell lines.[41,42] The transcription of mRNA species differing in the 5′-untranslated regions allows the quantitative regulation of ST6Gal I expression, not only at the transcriptional level, but also at the translational level.[34] In fact, the hepatic transcript shows a higher translational efficiency *in vitro*.[43] Several post-translational modifications affecting enzyme activity have been described so far.[36–39] These data provide the picture of an enzyme encoded by a single gene whose expression is precisely regulated in a tissue- and stage-specific manner, owing to the existence of multiple regulatory mechanisms.[34] Consistent with ST6Gal mRNA expression, no statistically significant difference of regulators (H form, Y+Z form, and X form) in the placental tissues of women with and without preeclampsia was found.

Since we could not find any statistical difference in either ST6Gal I mRNA expression, its regulator, or ST6Gal I enzyme activity in the placental tissues of women with and without preeclampsia, we supposed that neither ST6Gal I mRNA expression nor enzyme activity are involved in the pathogenesis of preeclampsia. The possible alternative explanations may include: (1) the timing of the study was not appropriate because

trophoblast invasion with forming uterine–placental circulation is active during the first trimester or the first half of pregnancy; the ST6Gal I activity, which underlies the pathogenesis of preeclampsia, might be more important at that time, while the mRNA expression and enzyme activity of ST6Gal I of the placenta were studied at the end of gestation in this study, resulting in the negative report; (2) ST6Gal I might not be involved in the formation of uterine–placental circulation; in contrast, 1 or more of the other STs, such as ST6GalNAc I, II, III, IV, V and VI, ST3Gal I, II, III, IV, V and VI, and ST8Sia I, II, III, IV, V and VI might be involved in the pathogenesis of preeclampsia. The other possibility is that there is a real absence of sialylation change in the placental tissues of women with preeclampsia. The tumor invasion process and trophoblast implantation process might be different. The aberrant behaviors in tumor cells (altered ST6Gal I expression and enzyme activity) in malignant diseases, including trophoblastic cancer cell lines (JEG-3 and BeWo; 2 choriocarcinoma cell lines) may not occur in trophoblast implantation for establishing the uterine–placental circulation, because trophoblast implantation is a sophisticatedly controlled invasive process. All the above might have contributed to the negative result in this study. We hope that a future study will be able to expand on the other types of STs and study the sialylation of placental tissue to clarify the relationship between ST and trophoblast implantation on the normal and abnormal pregnancy status.

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References

1. Petretti T, Kemmner W, Schulze B, Schlag PM. Altered mRNA expression of glycosyltransferase in human colorectal carcinomas and liver metastases. *Gut* 2000;46:359–66.
2. Wang PH. Altered sialylation and sialyltransferase expression in gynecologic cancers. *Taiwanese J Obstet Gynecol* 2004;43: 53–63.
3. Thomas P. Cell surface sialic acid as a mediator of metastatic potential in colorectal cancer. *Cancer J* 1996;9:1–10.
4. Schauer R. Sialic acids and their role as biological masks. *Trends Biochem Sci* 1985;10:357–60.
5. Kim YJ, Kim KS, Kim SH, Kim CH, Ko JH, Choe IS, Tsuji S, et al. Molecular cloning and expression of human Galβ1, 3GalNacα2,3-sialyltransferase (hST3GalII). *Biochem Biophys Res Commun* 1996;228:324–7.
6. Fukuda M. Possible roles of tumor-associated carbohydrate antigens. *Cancer Res* 1996;56:2237–44.
7. Hakomori S. Tumor malignancy defined by aberrant glycosylation and shingo-(glyco)-lipid metabolism. *Cancer Res* 1996;56: 5309–18.
8. Dorudi S, Kinrade E, Marshall NC, Feakins R, Williams NS, Bustin SA. Genetic detection of lymph node micrometastases in patients with colorectal cancer. *Br J Surg* 1998;85;98–100.
9. Aubert M, Panicot L, Crotte C, Gibier P, Lombardo D, Sadoulet MO, Mas El. Restoration of α(1,2) fucosyltransferase activity decreases adhesive and metastatic properties of human pancreatic cancer cells. *Cancer Res* 2000;60:1449–56.
10. Hsu CC, Lin TW, Chang WW, Wu CY, Lo WH, Wang PH, Tsai YC. Soyasaponin-I modified invasive behavior of cancer by changing cell surface sialic acids. *Gynecol Oncol* 2005;96:415–22.
11. Yamamoto H, Oviedo A, Sweeley C, Saito T, Moskal JR. α2,6-sialylation of cell-surface N-glycans inhibits glioma formation *in vivo. Cancer Res* 2001;61:6822–9.
12. Schneider F, Kemmner W, Haensch W, Franke G, Gretschel S, Karsten U, Schlag PM. Overexpression of sialyltransferase CMP-sialic acid: Galβ1,3GalNAc-Rα6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. *Cancer Res* 2001;61:4605–11.
13. Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Tsai YC, Yuan CC. Altered mRNA expression of sialyltransferase in squamous cell carcinomas of the cervix. *Gynecol Oncol* 2001;83: 121–7.
14. Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Ng HT, Tsai YC, et al. Expression of sialyltransferase family members in cervix squamous cell carcinoma correlates with lymph node metastasis. *Gynecol Oncol* 2002;86:45–52.
15. Chen CL, Lee WL, Tsai YC, Yuan CC, Ng HT, Wang PH. Sialyltransferase family members and cervix squamous cell carcinoma *Eur J Gynaecol Oncol* 2002;23:514–8.
16. Wang PH. Altered glycosylation in cancer: sialic acids and sialyltransferase. *J Cancer Mol* 2005;1:73–81.
17. Hung JH, Ng HT, Pan YP, Yang MJ, Shu LP. Color Doppler ultrasound of spiral arteries in normal second-trimester pregnancies. *J Chin Med Assoc* 1997;59:289–94.
18. Robillard PY. Interest in preeclampsia for researchers in reproduction. *J Reprod Immunol* 2002;52:279–87.
19. Granger JP. Pathophysiology of hypertension during preeclampsia linking placental ischemia with endometrial dysfunction. *Hypertension* 2001;38:718–22.
20. Nemansky M, Schiphorst WE, Koeleman CA, Van den Eijnden DH. Human liver and human placenta both contain CMP-NeuAc:Galβ1-->4GlcNAc-R α2-->3- as well as α2-->6-sialyltransferase activity. *FEBS Lett* 1992;312:31–6.
21. Tsuji S. Molecular cloning and functional analysis of sialyltransferases. *J Biochem* 1996;120:1–13.
22. Wang PH, Lee WL, Lee YR, Juang CM, Chen YJ, Chao HT, Tsai YC, et al. Enhanced expression of alpha 2,6-sialyltransferase ST6Gal I in cervical squamous cell carcinoma. *Gynecol Oncol* 2003;89:395–401.
23. Wang PH, Lee WL, Juang CM, Yang YH, Lo WH, Lai CR, Hsieh SL, et al. Altered mRNA expressions of sialyltransferases in ovarian cancers. *Gynecol Oncol* 2005;99:631–9.
24. Janssens N, Janicot M, Perera T, Bakker A. Housekeeping genes as internal standards in cancer research. *Mol Diagn* 2004;8: 107–13.
25. Gentle A, Anastasopoulos F, McBrien NA. High-resolution semiquantitative real-time PCR without the use of a standard curve. *Biotechniques* 2001;31:502–7.
26. Simpson DA, Feeney S, Boyle C, Stitt AW. Retinal VEGF mRNA measured by SYBR Green I fluorescence: a versatile approach to quantitative PCR. *Mol Vis* 2000;6:178–83.
27. Hu YC, Wang PH, Yeh S, Xie C, Wang RS, Xu Q, Zhou X, et al. Subfertility and defective folliculogenesis in female mice lacking

androgen receptor. *Proc Natl Acad Sci USA* 2004;101: 11209–14.
28. Wang PH, Lo WL, Hsu CC, Lin TW, Lee WL, Wu CY, Tsai YC, et al. Different enzyme activities of sialyltransferases in gynecological cancer cell lines. *Eur J Gynecol Oncol* 2002;23:221–6.
29. Kurosawa N, Kojima N, Inoue M, Hamamoto T, Tsuji S. Cloning and expression of Gal_1,3GalNAc-specific GalNAc_2,6-sialyltransferase. *J Biol Chem* 1994;269:19048–53.
30. Shennan AH. Recent developments in obstetrics. *BMJ* 2003; 327:604–8.
31. Chang WW, Yu CY, Lin TW, Wang PH, Tsai YC. Soyasaponin I decreases the expression of α2,3-linked sialic acid on the cell surface and suppresses the metastatic potential of B16F10 melanoma cells. *Biochem Biophys Res Commun* 2006;341:614–9.
32. Wang PH. Altered sialylation and its role in gynecological cancers. *J Cancer Mol* 2006;2:107–16.
33. Fukushima K, Hara-Kuge S, Seko A, Ikehara Y, Yamashita K. Elevation of alpha2->6 sialyltransferase and alpha1->2 fucosyltransferase activities in human choriocarcinoma. *Cancer Res* 1998;58:4301–6.
34. Dall'Olio F. The sialyl-α2,6-lactosaminyl-structure: biosynthesis and functional role. *Glycoconj J* 2000;17:669–76.
35. Taniguchi A, Kaneta R, Morishita K, Matsumoto K. Gene structure and transcriptional regulation of human Galβ1,4(3) GlcNAc α2,3-sialyltransferase VI (hST3Gal VI) gene in prostate cancer cell line. *Biochem Biophys Res Commun* 2001; 287:1148–56.
36. Taniguchi A, Hasegawa Y, Higai K, Matsumoto K. Transcriptional regulation of human beta-galactosideα2,6-sialyltransferase (hST6Gal I) gene during differentiation of the HL-60 cell line. *Glycobiology* 2000;10:623–8.
37. Svensson EC, Conley PN, Paulson JC. Regulated expression of α2,6-sialyltransferase by the liver-enriched transcription factors HNF-1, DBP, and LAP. *J Biol Chem* 1992;267:3466–72.
38. Wang X, O'Hanlon TP, Young RF, Lau JT. Rat β-galactoside α2,6-sialyltransferase genomic organization: alternate promoters direct the synthesis of liver and kidney transcripts. *Glycobiology* 1990;1:25–31.
39. Grundmann U, Nerlich C, Rein T, Kemmner W. Enhanced activity of CMP-NeuAc:Gal β1-4GlcNAc: α2,6-sialyltransferase. *Nucl Acids Res* 1990;18:667–8.
40. Wen DX, Svensson EC, Paulson JC. Tissue-specific alternative splicing of the β-galactoside α2,6-sialyltransferase gene. *J Biol Chem* 1992;267:2512–8.
41. Wang X, Vertino A, Eddy RL, Byers MG, Jani-Sait SN, Shows TB, Lau JT. Chromosome mapping and organization of the human β-galactoside α2,6-sialyltransferase gene: differential and cell-type specific usage of upstream exon sequences in B-lymphoblastoid cells. *J Biol Chem* 1993;268:4355–61.
42. Lo NW, Lau JT. Transcription of the beta-galactoside α2,6 sialyltransferase gene in B lymphocytes is directed by a separate and distinct promoter. *Glycobiology* 1996;6:271–9.
43. Aasheim HC, Aas-Eng DA, Deggerdal A, Blomhoff HK, Funderud S, Smeland EB. Cell-specific expression of human beta-galactoside alpha 2,6-sialyltransferase transcripts differing in the 5′ untranslated region. *Eur J Biochem* 1993;213: 467–75.