Progesterone-regulated *B4galnt2* expression is a requirement for embryo implantation in mice

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Objective: To investigate *B4galnt2* gene regulation in the female mouse reproductive system (*B4galnt2* encodes an enzyme, β1,4-*N*-acetylgalactosylaminyltransferase II, that catalyzes the addition of GalNAc to glycoproteins via a β1,4 linkage).
Design: Experimental prospective study.
Setting: Research institute and university.
Animal(s): Outbred Institute for Cancer Research (ICR) mice.
Intervention(s): Subcutaneous injection of P/E_2; uterine tissues were collected after a 3-day injection period and were collected at different times during pregnancy.
Main Outcome Measure(s): Gene expression was measured by quantitative real-time polymerase chain reaction after hormonal treatment of ovariectomized mice or pregnant mice. Primary endometrial cell cultivation and a gene promoter assay were used for P regulation analysis. The small interfering RNA (siRNA) technique was used to assess the gene function in embryo implantation in vivo.
Result(s): Animal experiments, a primary endometrial cell cultivation assay, and a gene promoter assay indicated that *B4galnt2* is regulated positively by P and negatively by estrogen. *B4galnt2* was expressed in uterine tissue at peri-implantation (embryonic day 3.5) along with a sharp increase in placental P production at embryonic day 10.5, and declined as estrogen increased during pregnancy. Using the siRNA in vivo implantation assay, we have proved that *B4galnt2* participated in embryonic implantation during pregnancy in mice.
Conclusion(s): This study shows for the first time the expression of *B4galnt2* in pregnant mice and its regulation by P. We conclude that the naturally occurring up-regulation of *B4galnt2* during pregnancy contributes to normal embryo implantation but not to embryo development. (Fertil Steril® 2011;95:2404–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: β,1,4-*N*-acetylgalactosaminyltransferase, embryo implantation, estrous cycle, pregnancy, Sd^a antigen

Glycosylation is an important molecular aspect of reproductive physiology from sperm maturation to blastocyst implantation (1). The uterine glycoproteins undergo cyclic alteration during the physiologic cycle; glycosylation may play a regulatory role in the cross-talk between the embryo and the endometrium and thereby support embryo implantation. Naz and Rajesh (2) mentioned that gene knockouts in the female reproductive tract affected fertility and postfertilization embryonic development. This suggests that gene expressions in uterine tissue should correlate with embryonic progression and development. Our previous work showed that the predominance of the Lewis x/y epitope along with Neu5Acα2-6-sialylation found in mouse uterine fluid is related to sperm motility (3). Furthermore, Klisch et al. (4) showed that the Sd^a antigen is the predominant N-glycan in bovine pregnancy-associated glycoproteins. The specific glycosylation pattern on pregnancy-associated glycoproteins strongly suggests a functional role of carbohydrates during pregnancy. Current evidence strongly suggests the significance of glycosylation in reproductive physiology (5). It indicates that glycosyltransferases should be important to fertility.

B4galnt2 encodes an enzyme, β1,4-*N*-acetylgalactosylaminyltransferase II (Sd^a-β1,4GalNAcT II), that catalyzes the addition of GalNAc to glycoproteins via a β1,4 linkage and forms an Sd^a antigen [GalNAcβ1-4(NeuAcα-2-3)Galβ1-4GlcNAc] on glycoproteins. B4GALNT2 was first manifested in the guinea pig kidney (6), with the next investigation demonstrating the abundance of this protein in human colon tissue (7). The decrease of B4GALNT2 activity and *B4galnt2* gene expression has been found during oncogenetic processes in colon tissue (8). Furthermore, B4GALNT2 was also detected in uterine tissue in an immunohistochemical assay, according to antibody proteomics (9). To date, the regulation of *B4galnt2* gene is unclear, especially in uterus. This is the reason for our interest in investigating *B4galnt2* expression in the uterus. Furthermore, Kawamura et al. (10) postulated that B4GALNT2 may compete strongly with other glycosyltransferases for the acceptor to produce Sd^a determinants and change the critical role in biological function. As a means to understanding the function of the Sd^a antigen, we first attempted to elucidate the regulation of β1,4-*N*-acetylgalactosylaminyltransferase. Our desire to learn about the regulation of *B4galnt2* gene expression encouraged us to initiate the study. The purpose of the present study was to explore molecular effects on fertility.

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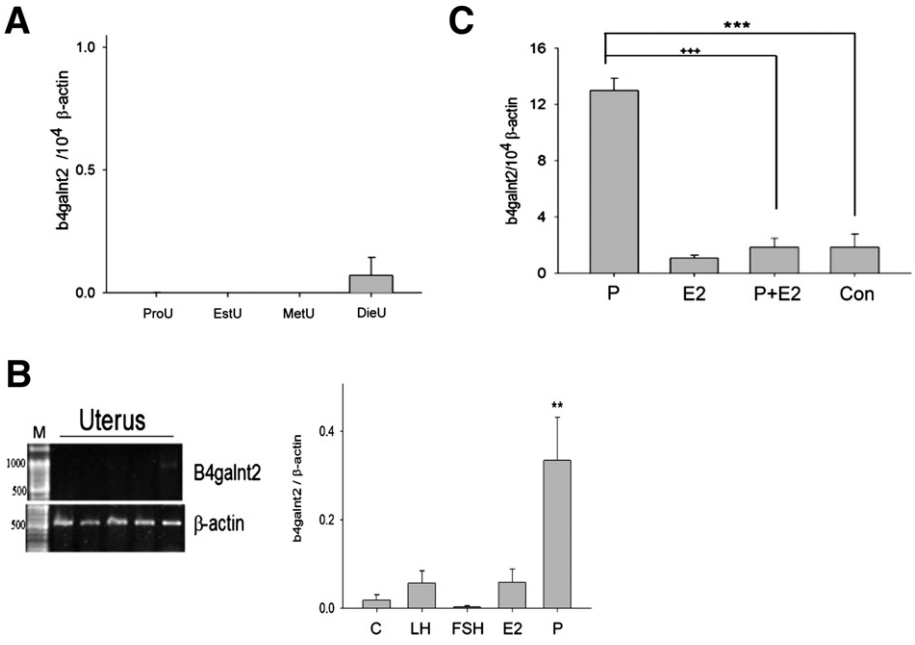
FIGURE 1

B4galnt2 expression in the uterus. (**A**) Quantitative PCR of *B4galnt2* in the mouse uterus during the estrous cycle at proestrous (Pro), estrous (Est), metestrous (Met), and diestrous (Die) stages. Amplification of *B4galnt2* was set to monitor the template cDNA. (**B**) Reverse transcription–PCR (RT-PCR) demonstrated the regulation of *B4galnt2* expression by sex hormones in the uterus of immature mice. The mice were treated with vehicle (**C**), LH (2.75 IU per mouse), FSH (2.5 IU per mouse), E_2 (30 ng/g), or P (150 μg/g) for 3 days, and RNA was then extracted from the tissues. The RT-PCR products are shown at left; densitometric analysis is shown at right. The relative amount of *B4galnt2* expression was normalized to the value of β-actin, and statistical analysis was performed using ANOVA, according to the data from four independent experiments. $^{**}P<.01$ vs. control. (**C**) Ovariectomized mice were treated with vehicle (Con), P (P; 150 μg/g body weight), E_2 (E2; 30 ng/g body weight), or a combination of P and E_2 (P + E2; 150 μg/g body weight + 30 ng/g body weight) for 3 days, and RNA was extracted from the oviduct and uterus. Statistical analysis was performed using ANOVA according to the data from four independent experiments. $^{***}P<.001$ vs. control; $^{+++}P<.001$ vs. P + E2.



Li. Gene regulation during estrous and pregnancy. Fertil Steril 2011.

To make it easier to examine the regulation of *B4galnt2* expression in the uterus, we used a mouse model as our study target.

MATERIALS AND METHODS

Experimental Animals

Outbred Institute for Cancer Research (ICR) mice were bred in the animal center at National Taiwan University, Taipei, Taiwan. Female mice were used for the experiments. The test animals were kept under controlled lighting (14 hours light/10 hours dark) at a constant temperature (23°C ± 2°C), with water and NIH-31 laboratory mouse food supplied ad libitum. The animal experiments were approved by the licensing committee of the animal center in Academia Sinica.

B4galnt2 Gene Expression

Total RNA was extracted from mouse tissues using TRI reagent (Molecular Research Center, Cincinnati, OH). Synthesis of complementary DNA (cDNA) was performed using a random primer and Moloney murine leukemia virus (MMLV) reverse transcriptase (Epicenter, Madison, WI). *B4galnt2* and β-actin forward and reverse primers (Supplementary Table 1) were used to amplify. Details are provided in Supplementary Text 1.

Cell Culture and Promoter Assay

For the promoter chloramphenicol acetyl transferase assay, the mouse Sertoli cell line (TM4; American Type Culture Collection, Manassas, VA) was used because of its existing estrogen (E) and P receptor (11). Cell culture and promoter assay are described in Supplementary Text 2.

Hormone Treatment in Animal and Primary Cultured Endometrial Cells

On the basis of our previous method (12), 20 6-week-old female ovariectomized mice were divided into five equal groups, and the 3-day hormone treatment was initiated. Details are provided in Supplementary Text 3.

Immunohistochemistry and Western Blotting

These methods are described in Supplementary Text 4.

Implantation Analysis In Vivo

Three adult female ICR mice were caged with a male mouse for mating. On day 3.5 and day 10.5 after pregnancy, the uterine horn was injected with Mock vector or RNA interference (RNAi)-containing vector by liposome. The manipulated method was followed as specified in the description of Zhang et al. (13). Vectors were obtained from the National RNAi Core Facility (Academia Sinica, Taiwan) and prepared in accordance with standard protocols. Cells were transfected with pLKO.1-TRCN0000093659–63 (indicated as RNAi59-63).

Statistical Analysis

Statistical analysis was conducted using one-way ANOVA with Dunnett's post-test using GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego, CA).