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Osteopontin increases the expression of β1, 4-Galactosyltransferase-I and promotes adhesion in human RL95-2 cells

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Abstract Beta1, 4-Galactosyltransferase-I (β1, 4-GalT-I), which transfers galactose from UDP-Gal to *N*-acetylglucosamine and *N*-acetylglucosamine-terminated oligosaccharides of N- and O-linked glycans in a β(1-4) linkage, plays a critical role in cell adhesion, sperm-egg recognition, neurite growth, and tumor cell migration and invasion. Our previously experiments also show that β1, 4-GalT-I was up-regulated by estrogens and some important cytokines of embryo implantation especially Interleukin-1 (IL-1), TGF-α and Leukemia Inhibitory Factor (LIF) in endometrial cells. In the receptive phase human uterus, osteopontin (OPN) is the most highly up-regulated extracellular matrix/adhesion molecule/cytokine. In this study, we demonstrated the correlated expression of OPN and β1, 4-GalT-I in endometrium during early pregnancy, and recombinant human OPN (rhOPN) protein induced the β1, 4-GalT-I up-regulation in RL95-2 cells. Inhibition of MEK/ERK, PI3K/AKT and NF-κB suppressed rhOPN-induced β1, 4-GalT-I expression. In addition, rhOPN promoted the adhesion of blastocysts cells *in vitro* in β1, 4-GalT-I-dependent manner. Moreover, the adhesion is greatly inhibited when β1, 4-GalT-I was blocked with the specific antibody. Taken together, our data suggest that β1, 4-GalT-I provides a mechanism to bridge embryo to endometrium during implantation.

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Keywords beta1, 4-Galactosyltransferase-I · Osteopontin · Embryo implantation · Adhesion

Abbreviations

β1 4-GalT-I	β1, 4-Galactosyltransferase-I
ECM	Extracellular matrix
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GlcNAc	*N*-acetylglucosamine
IL-1	Interleukin-1
PBS	Phosphate bufferedsaline
RGD	Arg-Gly-Asp
rhOPN	Recombinant human osteopontin
MUC-1	Mucin1
UDP-Gal	Uridine diphosphate-galactose
ZP3	Zonapellucida glycoprotein

Introduction

Embryo implantation is a complex process, including apposition, adhesion, penetration, and trophoblast invasion. These steps only occur during the implantation window, in which blastocyst is accepted by the maternal endometrium through mediation by adhesion molecules, immune cells, cytokines, chemokines, growth factors, and so on. These molecules contact with each other, and form a hormones-immune molecules-cell factors-adhesion molecular network system. The initial adherence of the blastocyst to endometrium is a main part of successful implantation. In the endometrium, there are a variety of adhesion molecules expressed, synchronized with opening of implantation window, and are relevant to the receptivity of endometrium. Therefore, the adhesion molecules are necessary to establish the required physical interaction between blastocyst and endometrium [1–3]. A number of adhesion molecules play a role in the various stages

of blastocyst implantation including L-selectin, mucin1 (MUC-1), Osteopontin (OPN) [4, 5], and intercellular adhesion molecules1 (ICAM-1) [6, 7].

OPN also known as secreted phosphoprotein 1 (SPP1), is a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family [8]. It contains a Arg-Gly-Asp (RGD) sequence that binds to cell surface integrin to mediate the cell-cell and cell-ECM attachment and communication, cell migration and adhesion [8–11]. OPN was first described in bone matrix, and also detected in epithelial cells [12, 13] and in secretions of the gastrointestinal tract [14], kidneys [15], mammary gland [16], oviduct [17], uterus [18], placenta [5], and testes [19]. Moreover, OPN is persistently expressed on the maternal-fetal interface, and it is speculated that OPN play an important role in maintaining uterine-embryonic microenvironment [20]. Accumulating data suggest that OPN has multiple molecular functions, which mediate cell adhesion [21], chemotaxis [22], angiogenesis [23], prevention of apoptosis [24] and anchorage-independent growth of tumor cells [25, 26]. In particular, the role of OPN has been studied extensively in reproduction [4, 5, 27–30]. It is also revealed that OPN improves the efficiency of bovine *in vitro* embryo production and influences sperm capacitation [31], and acts as a potential mediator of implantation in mammals [31–33]. OPN was highly expressed in endometrium during "implantation window phase", which suggests that OPN is related with endometrial receptivity and embryo implantation [30, 34].

β1, 4-galactosyltransferase-I (β1, 4-GalT-I) is ubiquitously expressed in human tissues with the exception of the brain. It exists in two subcellular compartments where it performs two distinct functions: in the Golgi apparatus, β1, 4-GalT-I acts as biosynthetic enzymes, where they catalyze the transfer of galactose from uridine diphosphate-galactose (UDP-Gal) donors to terminal *N*-acetylglucosamine (GlcNAc) residues [35]; on cell surface, β1, 4-GalT-1 acts as a recognition and adhesion molecule participates in a number of cellular interactions, including neurite extension [36], sperm-egg interaction [37, 38], cell adhesion [39–42] and migration [39, 41, 43]. Although several aspects of its regulation and function have been extensively studied, there are still many uncharacterized features. The process of the embryos implantation is very similar to tumor invasion, so many biological factors participate in the tumor invasion also play a role in embryo implantation. We hypothesize that β1, 4-GalT-I may take part in embryo implantation, since previous data suggest that cell surface β1, 4-GalT-I involved in the progression of Gamete recognition, which functions in a lectin-like capacity by binding to N-terminal *N*-acetylglucosamine residues on zona pellucida glycoprotein (ZP3) oligosaccharides [37, 38]. Our previous experiments also show that β1, 4-GalT-I was upregulated by estrogens and some important cytokines of embryo implantation especially Interleukin-1 (IL-1), TGF-α and Leukemia Inhibitory Factor (LIF) in endometrial cells. In this study, we demonstrate that β1, 4-GalT-I was highly expressed in endometrium during implantation window, and OPN upregulated the expression of β1, 4-GalT-I in RL95-2 cells in a time- and concentration-dependent manner, and promoted the adhesion of blastocysts cells *in vitro* in β1, 4-GalT-I-dependent manner. The results presented in this study may provide a view of the mechanism of adhesion in embryo implantation.

Materials and methods

Antibodies and reagents

Recombinant human OPN protein (rhOPN) was purchased from R&D Systems. Mouse polyclonal anti-OPN, goat polyclonal anti-β1, 4-GalT-I, mouse anti-IκBα, rabbit anti-a-Raf and mouse anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology. Rabbit anti-AKT, ERK, p38, phospho-ERK1/2, phospho-AKT (Ser473), phospho-AKT (Thr308) and phospho-p38 antibodies were purchased from Cell Signaling Technology. HRP-conjugated anti-goat, anti-mouse, or anti-rabbit secondary antibody was purchased from Santa Cruz Biotechnology. U0126, LY294002, SB203580 and BAY 11-7082 was obtained from Beyotime. Trizol reagent was obtained from Invitrogen. β1, 4-GalT-I siRNA and Scrambled siRNA was purchased from Santa Cruz Biotechnology. X-tremeGENE siRNA Transfection Reagent was purchased from Roche. Dulbecco's modified eagle medium/F12 (1:1) medium, Opti-MEM, RPMI1640 and Fetal bovine serum (FBS) were purchased from Gibico.

Animals and tissue collection

All procedures involving animals were carried out in accordance with the Guiding Principles for the Care and Use of Research Animals for the Study of Reproduction filed by the State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences. Mature C57 mice (6–8 weeks old) were purchased from the Experimental Animal Center of Institute of Zoology, Chinese Academy of Sciences. The mice were bred randomly under a controlled environment with a photoperiod of 12 h light and 12 h dark and a temperature ranging from 20–25 °C. To set up mating, two female mice were caged with a male overnight. The day of the vaginal plug being found was designated as Day 1 of pregnancy and so forth. Non-pregnant female mice were denoted as Day 0 as a control. To collect uteri, pregnant female mice were sacrifice on Days 1–5 of pregnancy.

Cell lines and cell culture

Two cell lines were used as *in vitro* models of implantation. Cell line human endometrial carcinoma RL95-2 was used as

a model of receptive endometrium, because of its high adhesiveness for trophoblast-derived cell [44–46]. JAR choriocarcinoma cell served as an *in vitro* model for the trophoblast cell [47]. All cell lines were purchased from the American Type Culture Collection (ATCC). The RL95-2 cells were cultured in Dulbecco's modified eagle medium/F12 (1:1) medium supplemented with 10 % FBS, 5 μg/ml insulin. Human JAR cells were maintained in monolayer culture in RPMI1640 supplemented with 10 % FBS. All cell line media were additionally supplemented with 1 % penicillin-streptomycin (100U/ml-100 μg/ml). Cell cultures were maintained in a humidified atmosphere containing 5 % CO2 95 % air at 37 °C.

Immunoblotting

For β1, 4-GalT-I expression, RL95-2 cells were treated with rhOPN in a time- and dose-dependent manner. In separate experiments, RL95-2 cells were incubated with rhOPN in different time or dose and level of β1, 4-GalT-I was detected. For kinase inhibition experiments, the cells were pretreated with 20 μM U0126 or 50 μM LY294002 or 50 μM SB203580 or 50 μM BAY 11-7082 for 1 h and then treated with 200 ng/ml rhOPN. The cells were lysed in SDS lysis buffer. The supernatant lysates containing equal amount of total protein were separated by SDS-PAGE and electrotransfered to a PVDF membrance (Roche). The membranes were incubated with primary and secondary antibodies. Anti-GAPDH antibody was used to confirm the equal loading.

RNA isolation and real-time PCR

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. The cDNA was synthesized using a RNA PCR Kit (AMV) version 3.0 (Takara). Real-time PCR was performed with an ABI Step One Plus Real-time PCR system (Applied Biosystems) according to the manufacturers' recommendations. Real-time PCR reaction contained 10 μl 2× SYBR Premix Ex Taq (TaKaRa), 0.8 μl primer mix, 0.4 μl 50× ROX Reference Dye II, 4 μl cDNA, and 4.8 μl deionized water to make a total volume of 20 μl. The relative amount of specific mRNA was normalized to GAPDH. All PCR reactions were run in triplicate and were performed with 40 cycles. The results analysis was carried out using the $2^{-\Delta\Delta Ct}$ method. The primers used were as follows. Mouse β1, 4-GalT-1: 5′-AGC AAC TCG ACT ATG GCA TC-3′ (forward), and 5′-AGG TCC ACA TCA CTG AAC AC-3′(reverse); mouse OPN: 5′- AGT GGT GCC AAG AGT GTG TT -3′ (forward), and 5′-AGG CAA TGC CAA ACA GGC AA-3′ (reverse); mouse GAPDH: 5′-TGT GTC CGT CGT GGA TCT GA-3′ (forward), and 5′-TTG CTG TTG AAG TCG CAG GAG-3′ (reverse); Human β1, 4-GalT-1: 5′-GAG GTG TCT CTG CTC TAA GTA AAC-3′ (forward), and 5′-TCC CGA CCA CAG CAT TTG-3′ (reverse); Human GAPDH: 5′-GTG AAG GTC GGA GTC AAC G-3′ (forward), and 5′-TGA GGT CAA TGA AGG GGT C-3′(reverse).

Transient transfection

Small-interfering RNA (siRNA) transfected into cells by using X-tremeGENE siRNA Transfection Reagent (Roche) at concentration of 0–15 nmol siRNA plus 4 μl X-tremeGENE siRNA Transfection Reagent in a six-well plate format with a final volume of 2 ml. 72 h after the siRNA treatment, the silencing efficiency was tested by Western blot.

Immunohistochemistry

Paraffin-embedded tissue specimens were obtained for immunohistochemical studies from surgical patients at the Second Affiliated Hospital of Dalian Medical University and Dalian maternity hospital. All the paraffin-embedded tissue specimens were confirmed by the Clinicopathologic Department at the Second Affiliated Hospital of Dalian Medical University. Tissue sections were de-paraffinized using xylene and re-hydrated in a series of alcohols. The tissue sections were then incubated at room temperature (RT) in 3 % H_20_2 for 15 min to inactivate endogenous peroxidase. Following incubation the slides were washed 3 times in PBS for 5 min each. Antigen retrieval was obtained by heating in a microwave in containing 6.5 mmol/l sodium citrate at maximum heat for 10 min. Slides were again washed 3 times. The slides were blocked 2 h in a humid chamber at RT with serum to reduce non-specific binding. Serum was removed from the slides and the slides were then incubated with the primary antibody β1, 4-GalT-I or OPN. After washing, the slides were incubated with a secondary antibody. After washing, the slides were incubated with HRP for 30 min in a humid chamber at RT. A 3, 3′diaminobenzidine (DAB) substrate was used for detection and hematoxylin was used for counterstain. The slides were then dehydrated and mounted. The slides were digitized to 20× magnification.

Attachment of JAR cells to the RL95-2 cell monolayer

In separate experiments, RL95-2 cells were transfected with 15 nmol β1, 4-GalT-I siRNA/scrambled siRNA or pretreated with 15 nmol β1, 4-GalT-I siRNA/scrambled siRNA and then treated with 200 ng/ml rhOPN. Before JAR cells were delivered, the monolayer of RL95-2 cells was monitored under the phase-contrast microscope to make sure there was no empty space. And JAR cells were traced with the fluorescent vital dye CFSE (Invitrogen) for 20 min at 37 °C and subsequently delivered onto confluent monolayers of endometrial RL95-2 cells. After 60 min of co-culture in JAR medium, un-adhered

JAR cells to the RL95-2 cell monolayer were removed by centrifugation for 5 min. Unattached cells were counted by Flow cytometry and the results expressed as the percentage of the number of cells seeded initially. Experiments were performed in duplicate and repeated three independent times. In antibody blockade experiment, RL95-2 cells were incubated with an antibody directed against β1, 4-GalT-I (0.2 mg/well).

Statistical analysis

All experiments were repeated at least three times. All data were presented as means ± SD. Statistic evaluations were achieved by Student's t-test. $P<0.05$ was considered statistically significant.

Results

Correlated expression of OPN and β1, 4-GalT-I in mouse endometrium during early pregnancy

To examine the expression patterns of OPN and β1, 4-GalT-I in the endometrium during early pregnancy, endometrium tissue on Days 1 to 5 of pregnancy were collected. Western blot assay revealed that the expression of OPN and β1, 4GalT-I slightly increased from Day 1 to Day 4, and showed a strong expression on Day 4 (the implantation window), followed by a decline thereafter (Fig. 1a). Real-time PCR also demonstrated similar results that the expression of OPN and β1, 4-GalT-I mRNA reached a peak at pregnant Day 4, after which the expression began to decline (Fig. 1b). Moreover, the expression of OPN pattern correlated well with that of β1, 4-GalT-I in mouse endometrium during early pregnancy. Since mice blastocyst implantation is in the fourth day of pregnancy, the results suggest that the molecules of β1, 4-GalT-I and OPN might be involved in the embryo implantation.

OPN induces β1, 4-GalT-I expression in RL95-2 cells

To determine whether OPN induces β1, 4-GalT-I expression, RL95-2 cell was treated without or rhOPN for 0–48 h and the expression of β1, 4-GalT-I in cell lysates was detected by western blot. Results indicated that rhOPN induces β1, 4-GalT-I expression in time-dependent manner in RL95-2 cells, and β1, 4-GalT-I reached maximum expression at the time point of 12 h (Fig. 2a). The dose-dependent response of rhOPN (0–400 ng/ml) on β1, 4-GalT-I expression was also detected. Results showed that the expression of β1, 4-GalT-I increases in dose-dependent manner, and 200 ng/ml rhOPN induced highest level of β1, 4-GalT-I expression compared with other doses (Fig. 2b). The result of real-time PCR showed that the mRNA level of

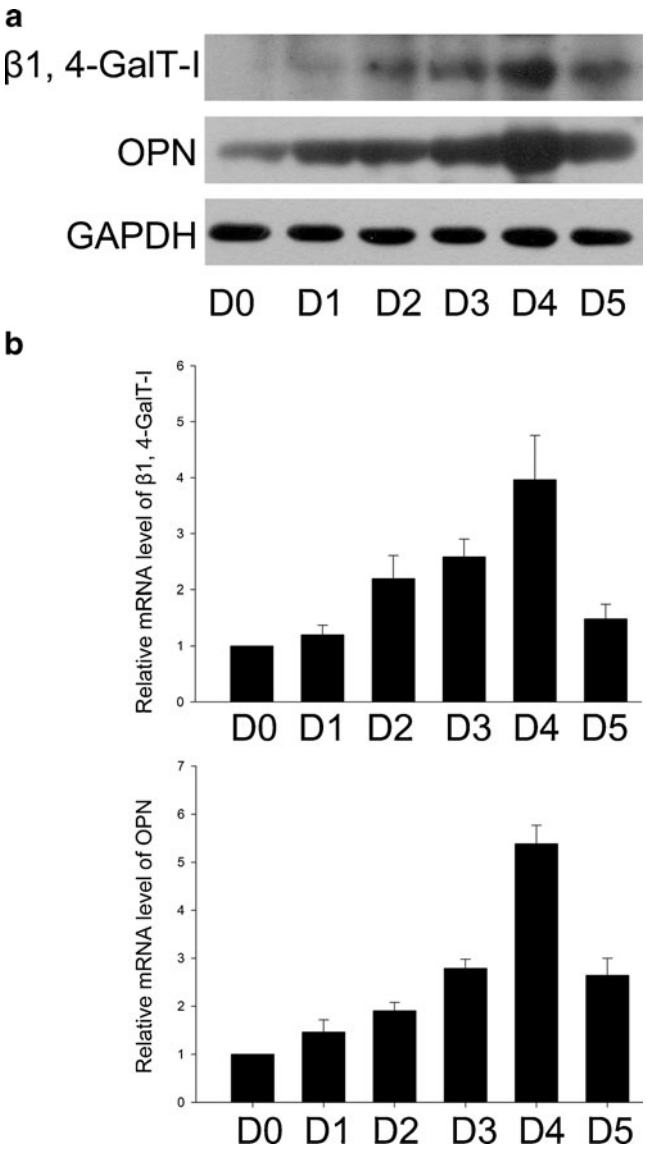

Fig. 1 The expression of OPN and β1, 4-GalT-I in early pregnancy in mice endometrium. The expression of OPN and β1, 4-GalT-I in protein and mRNA levels were detected by Western-blot (**a**) and Real-time PCR (**b**)

β1, 4-GalT-I treated with 200 ng/ml rhOPN for 4 h was about 2.7-times more than untreated cells (Fig. 2c and d).

OPN induces β1, 4-GalT-I expression in ERK1/2, AKT and NF-κB signaling-dependent manner

In order to examine how rhOPN up-regulated the expression of β1, 4-GalT-I, we measured the activation of ERK1/2, AKT, p38 and NF-κB activation upon rhOPN treatment. Results demonstrated that the level of a-raf, phospho-ERK1/2, phospho-AKT (thr308), phospho-p38, degradation of IκBα after rhOPN treatment increased in a dose-dependent manner (Fig. 3a). The activation of AKT (ser473) was not significantly different between rhOPN-

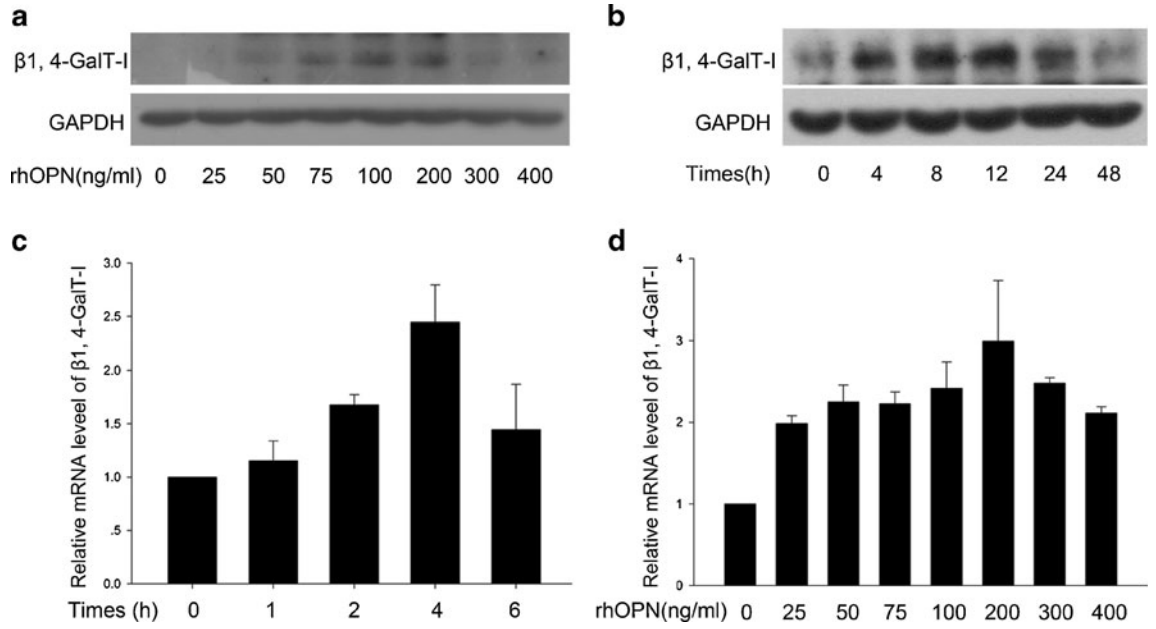

Fig. 2 rhOPN-induces β1, 4-GalT-I expression in RL95-2 cells. Western blot analysis β1, 4-GalT-I expression in RL95-2 cells after treatment with or without rhOPN (a and b). RL95-2 cells were treated with various concentrations of rhOPN (0-400 ng/ml) for 24 h (**a**), RL95-2 cells were treated with 200 ng/ml rhOPN for 0–48 h (**b**). Real-time PCR analysis of β1,4GalT-I mRNA in RL95-2 cells after treatment with rhOPN (C and D). RL95-2 cells were treated with 200 ng/ml rhOPN for 0–6 h (**c**), RL95-2 cells were treated with various concentrations of rhOPN (0-400 ng/ml) for 4 h (**d**)

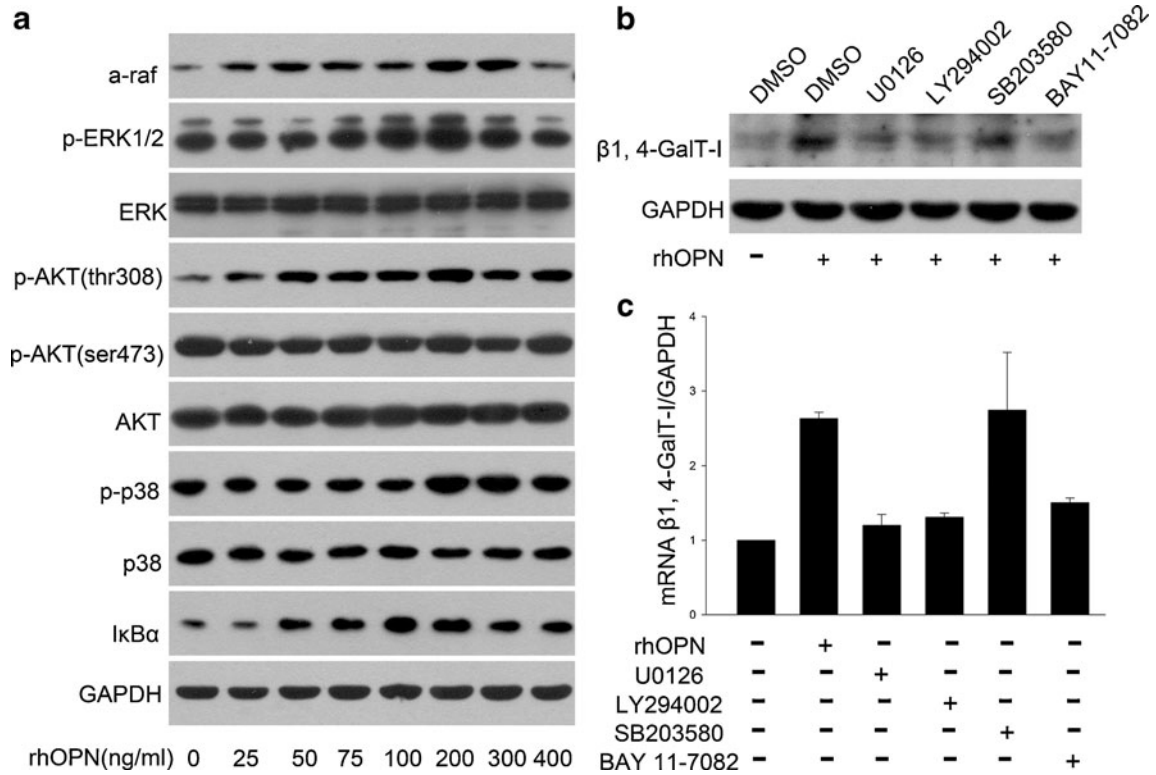

Fig. 3 OPN induces β1, 4-GalT-I expression through activating ERK1/2, p38, AKT (thr308) and degrading IκBα. RL95-2 cells were starved for 24 h then treated with rhOPN (**a**). Expression of a-raf, p-Erk, total Erk, p-AKT, total AKT, p-p38, total p38 and IκBα were assessed by Western blot. RL95-2 cells were respectively treated with Erk inhibitors (20 μmol/l U0126), AKT inhibitors, (50 μmol/l LY294002), p38 inhibitors (50 μmol/l SB203580) and NF-κB inhibitors (50 μmol/l BAY 11–7082) for 1 h before addition of rhOPN (b and c). The protein level (**b**) and mRNA level (**c**) were assessed

treated and control cells (Fig. 3a). To further study the role of signaling activation in rhOPN-induced β1, 4-GalT-I expression, the addition of U0126 (a specific inhibitor of MEK/ERK), LY294002 (a specific inhibitor of PI3K/AKT), SB203580 (a specific inhibitor of P38) and BAY (a specific inhibitor of NF-κB) was used to pretreat cells, respectively. Results showed that inhibition of ERK1/2, AKT and NF-κB signaling suppressed rhOPN-induced β1, 4-GalT-I expression, while inhibition of p38 showed little effect (Fig. 3b). Real-time PCR showed the similar results (Fig. 3c). These results indicated that rhOPN induces β1, 4-GalT-I expression possibly through activating ERK1/2, AKT (thr308) and degrading IκBα.

Effect of down-regulated β1, 4-GalT-I or antibody blockade of β1, 4-GalT-I expression on attachment of JAR cells to the RL95-2 cell monolayer

Two cell lines were used as *in vitro* models for implantation. Cell line RL95-2 was used as a model of receptive endometrium, because of its high adhesiveness for trophoblast-derived cells. And JAR choriocarcinoma cells serve as an *in vitro* model for the trophoblast cells. Using the *in vitro* implantation model, we found that rhOPN significantly facilitated the adhesion ability of JAR cells to the RL95-2 cell monolayer compared with control group (Fig. 4b and c). However, down-regulation of β1, 4-GalT-I suppressed

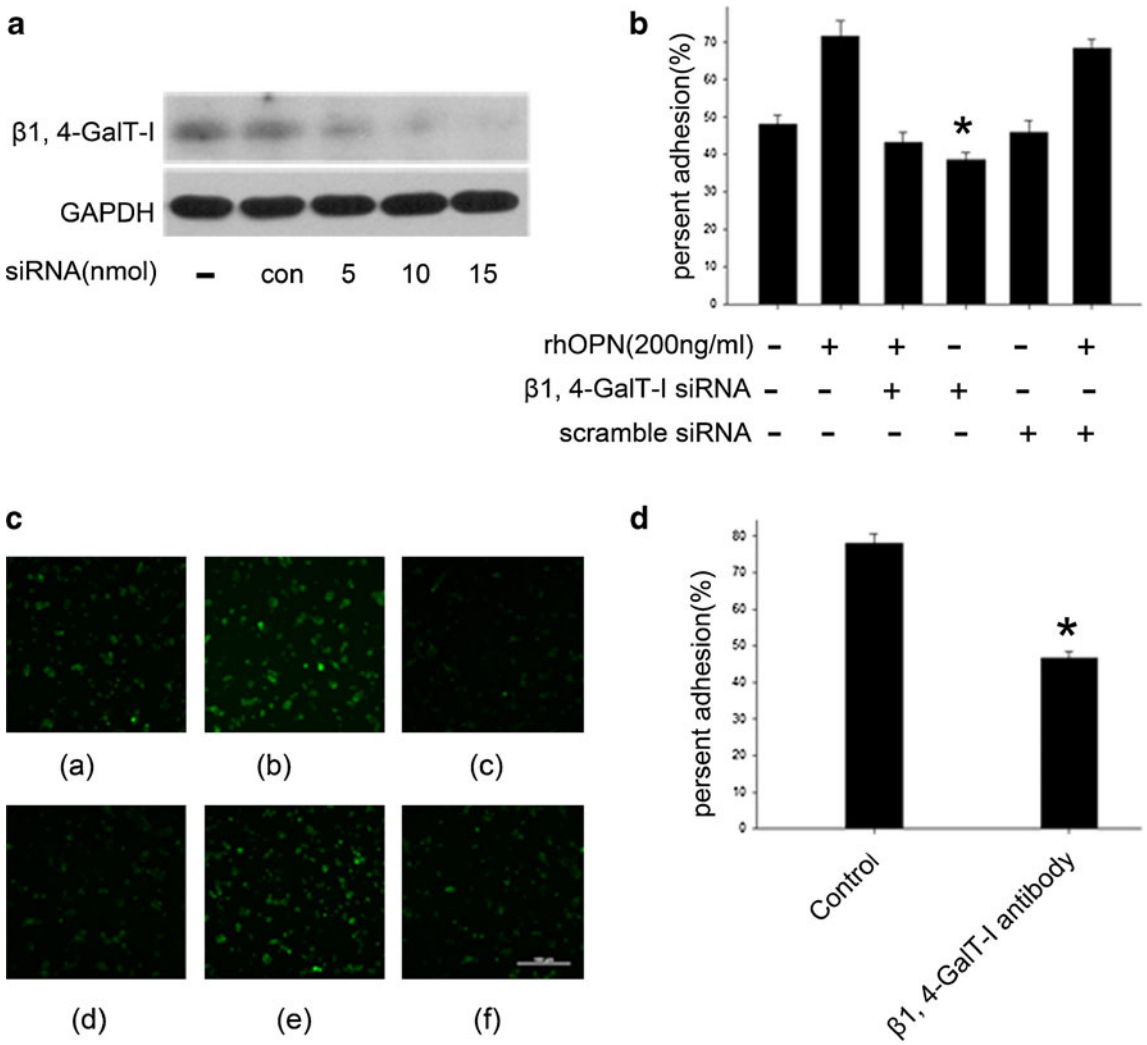

Fig. 4 Abolishing the expression of β1, 4-GalT-I prevents the adhesion of JAR cells to the RL95-2 cells. Western blotting of β1, 4-GalT-I protein isolated from RL95-2 cells transient transfected siRNAs against β1, 4-GalT-I (β1, 4-GalT-I siRNA), control siRNA (scrambled siRNA), or mock-transfected cells (**a**). RL95-2 cells were transfected without (control) or with their specific siRNA then co-colture with or without rhOPN, JAR cells were traced with the fluorescent vital dye CFSE before JAR cells were delivered to RL95-2 cells. Attached JAR cells were calculated by Flow cytometry (**b**). Attached JAR cells were detected by fluorescence microscope (**c**): RL95-2 cells without any treatment (*a*), RL95-2 cells co-culture with rhOPN (*b*), RL95-2 cells transfected with β1, 4-GalT-I siRNA (*c*), RL95-2 cells transfected with β1, 4-GalT-I siRNA then co-culture with rhOPN (*d*), RL95-2 cells transfected with scrambled siRNA then co-culture with rhOPN (*e*), RL95-2 cells transfected with scrambled siRNA (*f*). β1, 4-GalT-I on the RL95-2 cells were blocked without (control) or with their specific antibodies (**d**)

rhOPN-induced adhesion of JAR cells to the monolayer of RL95-2 cells. These results suggest that the increased adhesion ability induced by OPN may be due to the up-regulation of β1, 4-GalT-I.

To study whether blocking β1, 4-GalT-I affects attachment of JAR Cells to the RL95-2 Cell monolayer. β1, 4-GalT-I antibody was used to blockade of β1, 4-GalT-I. Results show that the antibody blockage of β1, 4-GalT-I decreases the trophoblast like JAR cell adhesion to the RL95-2 cells (Fig. 4d).

OPN and β1, 4-GalT-I expression pattern in human endometrium

We also analyzed expression patterns in human endometrium section. Using anti-OPN and anti-β1, 4-GalT-I antibodies in immunohistochemistry experiments, we detected constitutive expression of OPN and β1, 4-GalT-I in cells surface and surrounding gland, and in secretory phase endometrium the expression was significantly higher than proliferative phase endometrium (Fig. 5). The expression of OPN pattern also correlated with that of β1, 4-GalT-I 1 in human endometrium.

Disscusion

To date, studies show that some glycosyltransferases are localized on the plasma membrane in addition to their conventional Golgi location, a wide variety of observations confirm the presence of a few specific glycosyltransferases on the cell surface, most notably β1, 4-GalT-I [39, 41, 42, 48, 49]. It was hypothesised that glycosyltransferases may function as cell adhesion molecules by binding their complementary oligosaccharide substrates on adjacent cell surfaces or in the extracellular matrix [42]. It has been reported that surface-localized β1, 4-GalT-I serves as a laminin receptor to mediate specific cell-cell and cell-matrix interactions by binding to N-linked oligosaccharides in the E8 domain of laminin [50]. Also the surface β1, 4-GalT-I functions as a receptor for oligosaccharide ligands in the extracellular matrix, most notably during sperm binding to the egg coat and during cell interactions with the basal lamina [37, 41]. A great many of researches suggested that β1, 4-GalT-I is involved in tumor cells migration, adhesion and invasion [39, 40], extracellular matrix degradation [51]. Because the similarity between embryos implantation and

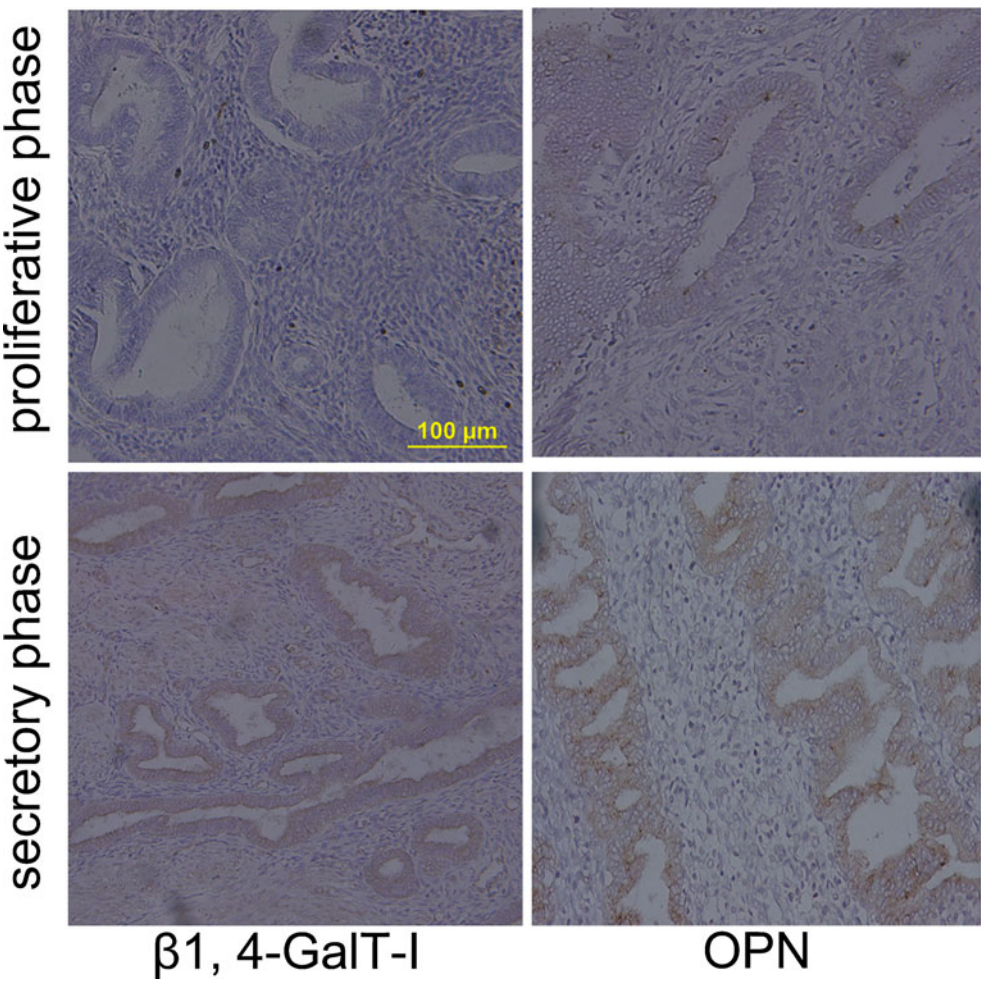

Fig. 5 OPN and β1, 4-GalT-I expression pattern in human endometrium. OPN and β1, 4-GalT-I were expressed at the cell surface and surrounding gland. The expression in secretory phase endometrium was significantly higher than proliferative phase endometrium

tumor invasion, we hypothesize that β1, 4-GalT-I may play a role at the conceptus-maternal interface. And so far there have been few studies on the expression and functions of β1, 4-GalT-I in embryo implantation. In the study, we found that β1, 4-GalT-I mRNA and protein expression in mouse endometrium epithelial cells of pregnancy Day 4 were both strongly high. For blastocysts adhered to the uterine epithelial cells occurred at Day 4 in pregnant mice (the implantation window). It is revealed that β1, 4-GalT-I might participate in the process, which endometrium changes to the receptive state to accommodate blastocyst implantation.

Osteopontin(OPN) is an extracellular matrix (ECM) molecule, multifunction, phosphorylated glycoprotein synthesized by many cell types and involved in many physiologic and pathologic processes, including cell adhesion, angiogenesis, inflammatory responses, and tumor metastasis [18, 25, 52]. The transcription of OPN is Increased induced by interleukin (IL), transforming growth factor β-1 (TGFβ-1), fibroblast growth factor (FGF), tumor necrosis factor α (TNFα), interferon γ (IFNγ), estrogen, progesterone, glucocorticoids [53–59]. On reproduction, secreted OPN was available as ligand for integrin αvβ3 heterodimer on trophectoderm and uterus to induce adhesion between luminal epithelium and trophectoderm essential for implantation and placentation [5, 8, 60, 61]. OPN is hypothesized to influence the uterine environment as 1) a component of histotroph required for adhesion and signal transduction at the uterine-placental interface, 2) a gene product expressed by uterine stroma as it decidualizes in response to conceptus invasion, and 3) a product of resident placental and uterine immune cells that regulates their behavior and cytokine production [5].

In this experiment, we focused on the possible association between β1, 4-GalT-I and OPN in embryo implantation. To our knowledge, there are no data on the relationship between β1, 4-GalT-I and OPN. We found that β1, 4-GalT-I and OPN were both highly expressed in mouse endometrium during the implantation window and immunohistochemistry results demonstrated OPN and surface-localized β1, 4-GalT-I were secreted by glands of secretory phase endometrium. It is consistent with previous reports that OPN is expressed in endometrium in the mid and late secretory phases, and our data also revealed that β1, 4-GalT-I and OPN are coordinately expressed with similar tendency in human endometrium and mice endometrium in the mid and late secretory phases (Figs. 1a and 5). It is suggested β1, 4-GalT-I and OPN were involved in the progression of implantation.

Adhesive interactions of trophoblast cells with the endometrium are essential for embryo implantation in the uterus. In this study, we used two cell lines as *in vitro* models of implantation model. Cell line RL95-2 was used as a model of receptive uterine epithelium, JAR choriocarcinoma cells serve as an *in vitro* model for the trophoblast cells. Because RL95-2 as a human uterine epithelial cell line that exhibits adhesion competence on its apical surface for trophoblast-like JAR cells [45–47, 62]. In contrast, the other human uterine epithelial cell line HEC-1-A cell line, which displays poor adhesive properties for trophoblast cells, is considered to be less receptive. On the other hand, JAR Choriocarcinoma malignant cells derived from human trophoblast the first trimester placenta, cultured *in vitro* retain several characteristics of the placental trophoblast [44]. The cell line show pronounced invasiveness and are of interest for model studies. *In vitro* models of implantation model, we found that β1, 4-GalT-I was up-regulated by OPN facilitated the adhesion ability of JAR cells to the RL95-2 cell monolayer. But the precise mechanism(s), especially the downstream effects of the surface-localized β1, 4-GalT-I in the adhesion response, needs further investigation.

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