FUT7 antisense sequence inhibits the expression of FUT7/sLeX and adhesion between embryonic and uterine cells

ARTICLE *in* INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY LIFE · JULY 2008

Impact Factor: 3.14 · DOI: 10.1002/iub.62 · Source: PubMed

6 AUTHORS, INCLUDING:

Xiao-qi Wang
Northwestern University
42 PUBLICATIONS **933** CITATIONS

SEE PROFILE

IUBMB *Life*, 60(7): 461–466, July 2008

Research Communication

FUT7 Antisense Sequence Inhibits the Expression of FUT7/sLeX and Adhesion Between Embryonic and Uterine Cells

Shuai Liu[1], Yuanyuan Zhang[1], Yuejian Liu[1], Huamin Qin[1], Xiaoqi Wang[2] and Qiu Yan[1]

[1]*Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Lab of Glycobiology and Glycoengineering, Dalian, People's Republic of China*

[2]*Department of Dermatology, Northwestern University's Feinberg School of Medicine, Chicago, IL, USA*

Summary

Implantation is a complex developmental event that is initiated by recognition and adhesion of the embryo to the endometrial epithelium. sLeX is an oligosaccharide antigen acting as the ligand of L-selectin, and is stage-specifically expressed in the endometrial epithelium. The adhesion system mediated by L-selectin and sLeX oligosaccharide plays an important role in this process. FUT7 is a key enzyme for sLeX synthesis, and the regulation of sLeX through FUT7 may influence maternal–fetal recognition. In this study, we observed the effect of FUT7 antisense oligodeoxynucleotide on the expression of FUT7 and sLeX, as well as adhesion in an *in vitro* implantation model consisting of the human uterine epithelial cell line RL95-2 and the human embryonic cell line JAR. Results showed that the expression of FUT7 was significantly decreased, compared with controls, after FUT7 antisense oligodeoxynucleotide transfection into RL95-2 cells, as determined by RT-PCR, Western blotting, and indirect immunofluorescence. Synthesis of sLeX was also decreased, consistent with the FUT7 decrease, as shown by indirect immunofluorescence. The adhesion of embryonic cells to uterine epithelial cells was significantly reduced ($P < 0.01$) compared with the control. These data indicate that the use of a FUT7 antisense oligodeoxynucleotide can cause a significant reduction of both FUT7 and sLeX antigen, and thereby inhibit the adhesion of embryo cells to endometrium. This approach may provide a new way to regulate reproduction.
IUBMB *Life*, 60(7): 461–466, 2008

Keywords antisense oligodeoxynucleotide; FUT7; sLeX; endometrium; embryo; implantation; glycobiology.

Received 22 November 2007; accepted 30 January 2008

Address correspondence to: Qiu Yan, Department of Biochemistry and Molecular Biology, Dalian Medical University, Liaoning Provincial Core Laboratory of Glycobiology and Glycoengineering, Dalian 116027, People's Republic of China. Tel.: +86-411-84722083. Fax: +86-411-84722083. E-mail: yanq63@126.com

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.62

INTRODUCTION

Embryo implantation is initiated by the recognition and adhesion of the embryo to the endometrial epithelium, and many adhesive molecules are involved the initial molecular interactions at the maternal–fetal interface (*1*). The oligosaccharides carried by the adhesive molecules, for example, sialyl Lewis X (sLeX), Lewis Y (LeY), and Lewis X (LeX), are stage-specifically expressed by the endometrial epithelium. They reach relatively a high level on the cell surface during the implantation stage (*2–5*). Sialyl Lewis X, the ligand of L-selectin, plays an important role through L-selectin-ligand adhesion system.

L-selectin-ligand adhesion system are involved in some physiological and pathological processes, for example, inflammation, tumor metastasis and embryo implantation (*6–8*). In the implantation stage, the human endometrium upregulates sLeX oligosaccharide synthesis, and on the fetal side, the expression of L-selectin increases in the trophoblasts (*9, 10*). It is considered that sLeX binds with L-selectin and mediates the initial recognition and adhesion between embryo and endometrium, which is crucial to establish human pregnancy (*1, 10*).

Sialyl Lewis X (NeuAcα2→3Galβ1 →4 [Fucα1-3] GlcNAcβ1→3Galβ1→R) is a fucosylated oligosaccharide. The last step of sLeX synthesis is catalyzed by the specific α1,3-fucosyltransferases (α1,3-FUTs). Currently, six kinds of α1,3-FUTs, FUT3, FUT4, FUT5, FUT6, FUT7 and FUT9, have been identified (*11–13*). Among these, the acceptor specificity of FUT7 is restricted to the distal *N*-acetylglucosamine on α2,3-sialylated lactosamines forming sLeX antigen, therefore, FUT7 is a key enzyme for the synthesis of sLeX (*14, 15*). The study shows that inhibition of FUT7 expression suppress the production of sLeX, thereby reducing carbohydrate-dependent cell adhesion in the endothelial cells (*16*), but the inhibitory effect of FUT7 on sLeX and embryo implantation remains unknown.

In this study, an *in vitro* implantation model composed of human uterine epithelial cell line (RL95-2) and human embryonic cell line (JAR) was utilized. RL95-2 cells and JAR cells can mimic the receptive uterine epithelium and adhesion-competent embryo, respectively (*17–19*). To inhibit the expression of FUT7 gene, an antisense oligodeoxynucleotide targeting specific FUT7 sequence was synthesized and transiently transfected to RL95-2 cells. We found that reduction of FUT7 expression by the antisense oligodeoxynucleotide was associated with the decreased synthesis of sLeX in RL95-2 cells, and the reduced adhesion of JAR embryonic cells to RL95-2 uterine epithelial cells.

MATERIALS AND METHODS

Antisense Oligodeoxynucleotides

An antisense oligodeoxynucleotide (5′-CAGCATTATT CATCCACAGT-3′, 19-38) and a control sense oligodeoxynucleotide (5′-ATGAATAATGCTGGGCACGG-3′, 26–45) were designed against the initiation region of the published sequence for human FUT7 (GenBank accession number: BC074746) by RNA Structure Software. Oligodeoxynucleotides were synthesized, purified and modified by phosphorothioate at the Shanghai Company (China). The specificity of the oligodeoxynucleotides was confirmed by BLAST sequence analysis.

Cell Culture

The human uterine epithelial RL95-2 cells and human embryonic JAR cells were obtained from the American Type Culture Collection (Manassas, VA). RL95-2 cells were grown in DMEM/F12(1:1) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 5 μg/mL insulin (Sigma–Aldrich, St. Louis, MO), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C under 5% CO_2 in humidified air. The JAR cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C under 5% CO_2 in humidified air according to standard procedures. The growth medium was renewed every 2–3 days.

Transient Transfection

RL95-2 cells (5×10^4/mL) were trypsinized and seeded onto six-well plates. When cells reached 90% confluence, the transfection was performed. The cells were transfected with 200 nM sense oligodeoxynucleotides, and either 50 nM, 100 nM, or 200 nM antisense oligodeoxynucleotides using 2 μL of Lipofectamine™ Reagent and Plus™ Reagent following the manufacturer's instructions (Invitrogen). An untransfected control was maintained. RNA and protein samples were obtained from RL95-2 cells after incubation for 48 h.

RT-PCR

Total RNA was extracted from cells using Trizol reagent (Invitrogen). cDNA was synthesized using a RNA PCR Kit (AMV) Version 3.0 (Takara, Otsu, Shiga, Japan). For PCR, the FUT7 gene forward primer was 5′-CACCTCCGAGCATCTT CAACTG-3′ and reverse primer was 5′-CGTTGGTATCGG CTCTCATTCATG-3′ and produced a PCR product of 497 bp. The β-actin gene forward primer was 5′-ATCTGGCACCA CACCTTCTACAATGAGCTGCG-3′ and reverse primer was 5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′ and produced a PCR product of 838 bp. PCR reactions to amplify the DNA fragment were carried out as follows: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 40 sec, 57°C for 45 sec, 72°C for 45 sec, and a final extension at 72°C for 5 min. The amplified products were analyzed by 1% agarose gel electrophoresis, and the bands were visualized by ethidium bromide staining, followed by analysis with LabWorks 4.6 gel imaging and analysis software (UVP. Inc). β-Actin expression was detected as the internal control. The experiments were repeated three times.

Western Blotting

To prepare whole cell protein extracts, cells were washed in PBS and resuspended in cell lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM Na_3VO_4, 0.2 mM phenylmethylsulfonyl fluoride, 0.5% Nonidet P-40) and incubated on ice for 30 min. The cell lysates were clarified by centrifugation at 9,000*g* for 10 min, and the supernatant was collected. Protein concentration was determined with the Coomassie Protein Assay Reagent (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard. Total protein lysates (80 μg) were separated by 10% SDS-PAGE mini-gel. Samples were transferred electrophoretically to nitrocellulose membranes. After blocking with TTBS (50 mM Tris-HCl pH 7.5, 0.15 M NaCl, 0.1% Tween-20) containing 5% fat-free dry milk for 2 h, the membrane was incubated with goat anti-FUT7 (1:300) (Santa Cruz Biotech, Santa Cruz, CA) in TTBS containing 5% fat-free dry milk for 2 h at 37°C. After washing with TTBS three times, 5 min each, the membrane was incubated with biotinylated anti-goat IgG (1:500) (Santa Cruz Biotech) antibody. After washing in TTBS, the bound antibody was detected through the use of avidin-conjugated horseradish peroxidase (1:500) (Santa Cruz Biotech). Color reaction was obtained using NBT/BCIP. The same blot was reprobed with anti-β actin antibody (Santa Cruz Biotech) to confirm equal loading. The membranes were scanned and analyzed using LabWorks 4.6 software. The experiments were repeated three times.

Indirect Immunofluorescence Staining

Transfected cells were also grown on glass coverslips. Forty-eight hours after transfection, the cells were washed with PBS and then fixed in 4% paraformaldehyde for 15 min. Cells were permeabilized with 0.1% Triton-PBS for 10 min at room temperature for FUT7 staining. After blocking with 1% complete serum for 2 h at 37°C, goat anti-FUT7 antibody (1:100) and mouse anti-sLeX antibody (1:100) (Chemicon, Temecula, CA) were applied to the slide and incubated overnight at 4°C. The

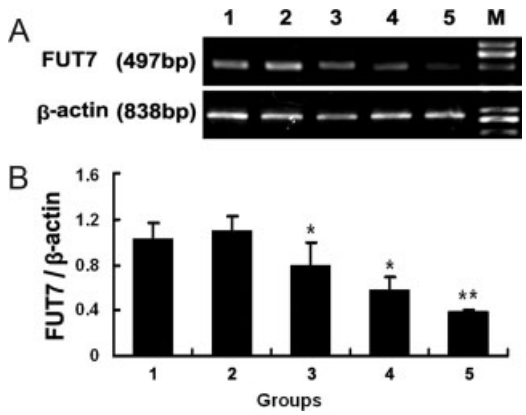

Figure 1. RT-PCR analysis of FUT7 gene. (A) FUT7 gene expression. (B) The relative density of FUT7 mRNA *vs.* β-actin mRNA. The lanes are: 1: untransfected control cells; 2: 200 nM sense oligodeoxynucleotide; 3: 50 nM antisense oligodeoxynucleotide; 4: 100 nM antisense oligodeoxynucleotide; 5: 200 nM antisense oligodeoxynucleotide (*$P < 0.05$; **$P < 0.01$).

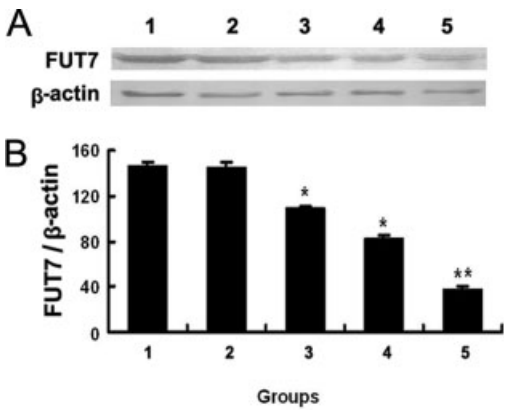

Figure 2. Western blotting analysis of FUT7 protein expression. (A) FUT7 enzyme level. (B) The relative density of FUT7 *vs.* β-actin. The lanes are: 1: untransfected control cells; 2: 200 nM sense oligodeoxynucleotide; 3: 50 nM antisense oligodeoxynucleotide; 4: 100 nM antisense oligodeoxynucleotide; 5: 200 nM antisense oligodeoxynucleotide (*$P < 0.05$; **$P < 0.01$).

coverslips were washed three times with PBS, and then incubated with FITC-conjugated anti-goat IgG (1:200) (Sigma) or TRITC-conjugated rat anti-mouse IgM (1:200) (Sigma) for 30 min at 37°C. After rinsing, specimens were mounted in PBS containing 90% glycerol and 1.0% *p*-phenylenediamine and subsequently monitored under a BX51 Olympus microscope.

Adhesion of JAR Cells to RL95-2 Cell Monolayer

The adhesion assay was performed as described (*19*). In brief, RL95-2 cells were grown on the coverslips to form confluent monolayers, and then differently treated before addition of JAR cells as follows: cells of untransfected (control); transfected with 200 nM sense oligodeoxynucleotides; 50 nM, 100 nM, or 200 nM antisenseoligodeoxynucleotides; preincubated with anti-sLeX antibody (5 μg/mL) for 30 min; and cells transfected with 200 nM antisense oligodeoxynucleotides followed by incubation with anti-sLeX antibody (5 μg/mL) for 30 min. Then, the cultured JAR cells (5×10^4) were harvested, counted, and gently delivered onto the confluent monolayer of RL95-2 cells in JAR growth medium. After 1 hr, JAR cells unadhered to the endometrial cell monolayer were removed by centrifugation of the coverslips with the cell surface facing down at 12*g* for 5 min. The unadhered cells are counted after resuspension of the pellet with PBS, and the attached JAR cells on the coverslip were also counted for further confirmation. The assay was repeated three times. Adhesion potential was calculated as the percentage of the JAR cells that had attached compared with the total number of JAR cells plated.

Statistical Analysis

Results are expressed as mean ± standard error of mean (SEM) of three independent experiments. Statistical significance of difference between test groups was assessed by one-way ANOVA followed by Scheffe's test (*post hoc*), with $P < 0.05$ considered to be significant.

RESULTS

FUT7 Gene Expression Detected by RT-PCR

To determine the effect of antisense oligodeoxynucleotides on FUT7 gene expression, different doses of antisense oligodeoxynucleotides were transiently transfected into RL95-2 cells. A semi-quantitative RT-PCR was adopted to analyze the expression of FUT7 mRNA (Fig. 1). The relative density of a 497 bp product of the FUT7 gene and an 838 bp product of internal control β-actin was calculated after separation by 1% agarose gel electrophoresis. Results showed that the expression of FUT7 gene was dramatically decreased in antisense oligodeoxynucleotide transfected RL95-2 cells in comparison with the untransfected control or sense oligodeoxynucleotide transfected cells. The inhibition of gene expression in the cells treated with 50 nM, 100 nM, or 200 nM antisenseoligodeoxynucleotides was 21.8%, 43.5%, and 60.2%, respectively, and in a dose dependent manner.

FUT7 Protein Expression Detected by Western Blotting

To detect the protein expression level of FUT7 in RL95-2 cells after oligodeoxynucleotide transfection, Western blotting was employed (Fig. 2). Results revealed that the expression level of FUT7 was much lower in antisense oligodeoxynucleotide transfected RL95-2 cells in comparison with the untransfected control or sense oligodeoxynucleotide transfected cells. The level of reduction was 26.2%, 44.2% and 74.0%, respec-

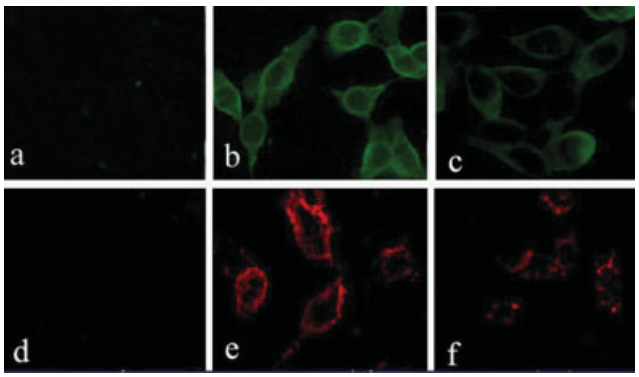

Figure 3. Indirect immunofluorescence analysis of FUT7 and sLeX expression in RL95-2 cells. Staining of FUT7 (a–c) was performed with anti-FUT7 primary antibody and FITC-conjugated anti-goat IgG secondary antibody and expression of sLeX (d–f) with anti-sLeX antibody and TRITC-conjugated rat anti-mouse IgM. a, d: negative control; b, e: untransfected control cells; c, f: 200 nM antisense oligodeoxynucleotide (Original magnification, ×400).

tively, with the strongest inhibition in 200 nM antisense oligodeoxynucleotide transfection. Results indicate that antisense oligodeoxynucleotide transfection effectively interferes with the expression of FUT7 and the effect is dose dependent.

Indirect Immunofluorescence Analysis of FUT7 and sLeX

Both FUT7 and sLeX synthesis in RL95-2 cells were analyzed after oligodeoxynucleotide transfection by indirect immunofluorescence. Results showed that FUT7 was mainly located in the cytoplasm (Figs. 3b and 3c) and sLeX on the cell membrane (Figs. 3e and 3f). The fluorescence intensity of both FUT7 and sLeX in the cells transfected with antisense oligodeoxynucleotide (200 nM) was weaker than that of the untransfected controls (Figs. 3c *vs.* 3b, 3f *vs.* 3e). This indicates that the synthesis of FUT7 and sLeX in RL95-2 cells is decreased after antisense oligodeoxynucleotide transfection.

Analysis of Adhesion

To observe the influence of antisense oligodeoxynucleotide on the adhesion of JAR cells to RL95-2 cell monolayer, the *in vitro* adhesion model of RL95-2 cells and JAR cells was used (Fig. 4A). Statistical analysis showed that the percent adhesion had no difference between the untransfected control (68.6% ± 1.5%) and sense oligodeoxynucleotide transfected cells (63.0% ± 2.1%). However, the percent adhesion in RL95-2 cells transfected with antisense oligodeoxynucleotides was significantly decreased compared with the untransfected control ($P < 0.01$), and the lowest percent adhesion of the cells transfected with 200 nM antisense oligodeoxynucleotide was 48.0% ± 2.0%. In addition, blocking the RL95-2 cells with anti-sLeX antibody caused a decreased percent adhesion (42.3% ± 2.4%)

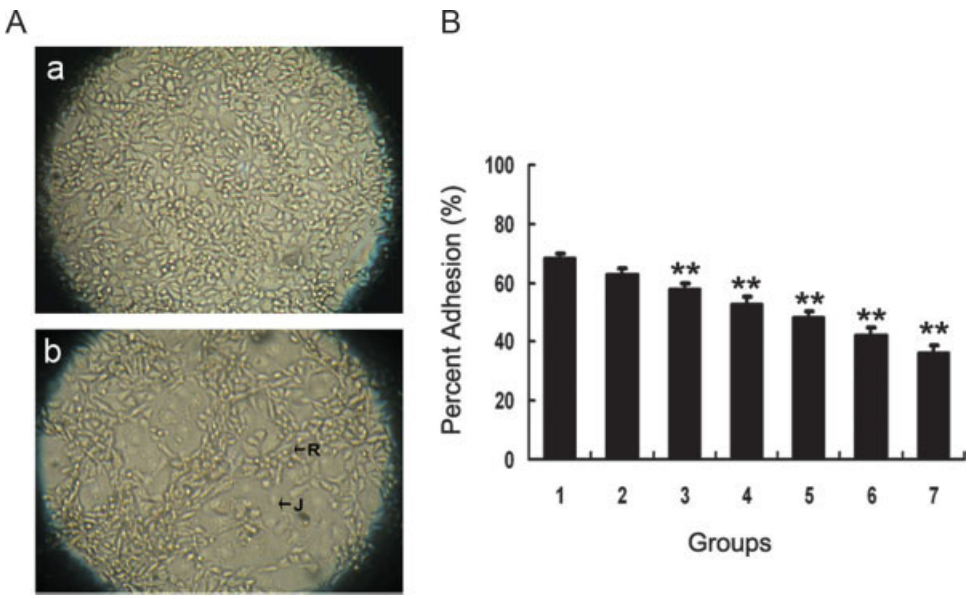

Figure 4. Analysis of adhesion of JAR embryonic cells to RL95-2 uterine epithelial cells. (A) Attachment of JAR cells on RL95-2 cells. a: RL95-2 cell monolayer; b: a representative image of the attached JAR cells on RL95-2 cells; R: RL95-2 cells; J: JAR cells; (B) percent adhesion was calculated as the percentage of attached JAR cells. The lanes show the results for:1: untransfected control cells; 2: 200 nM sense oligodeoxynucleotide; 3: 50 nM antisense oligodeoxynucleotide; 4:100 nM antisense oligodeoxynucleotide; 5: 200 nM antisense oligodeoxynucleotide; 6: RL95-2 cells incubated with anti-sLeX antibody; 7: RL95-2 cells transfected with 200 nM antisense oligodeoxynucleotide and incubated with anti-sLeX antibody (**$P < 0.01$).

compared with the untransfected control. The percent adhesion (36.4% ± 2.5%) was the strongest inhibited in the cells with 200 nM antisense oligodeoxynucleotide transfection followed by incubation with anti-sLeX antibody. The results indicate that antisense oligodeoxynucleotides of FUT7 gene inhibits the adhesion of embryo cells to the endometrial cells through decreased expression of FUT7 enzyme and sLeX antigen.

DISCUSSION

Implantation is a complex developmental process, and the cross-talking based on the molecular interactions between the mature embryo and receptive endometrium is crucial (*20, 21*). The elevated expression of the oligosaccharides carried by glycoconjugates are not only the receptive markers but also the functional adhesive ligands of the endometrial epithelium during implantation period. For example, LeY is highly expressed in the endometrial epithelium of human and mouse at implantation window, and preincubation of the cultured murine epithelium with anti-LeY antibody significantly blocks the adhesion and implantation of the embryo to the epithelium (*22*). Sialyl Lewis X expressed by the endometrium is also closely related to the functional state of implantation. Sialyl Lewis X of human endometrial epithelium increases in abundance after ovulation and reaches a maximum (*23*). It is also correlated with the further trophoblast emigration from the placenta (*24*).

The synthesis of stage-specifically expressed fucosylated oligosaccharides are controlled by their respective fucosyltransferases, the key synthesis enzymes. FUTs are grouped as α1, 2-, α1, 3- and α1, 6- FUTs according to the types of fucosylation linkage (*11,12*). FUT1 and FUT4 take part in the synthesis of LeY oligosaccharide antigen (*25*), and FUT9 is the key enzyme for LeX production (*26, 27*). FUT7 is mainly expressed in leukocytes, endothelial cells (*15, 28*) and tumor cells (*29, 30*). FUT7 synthesizes only sLeX epitope (*14*). Transfection of FUT7 cDNA can increase the synthesis of sLeX antigen (*31*). Recently, we introduced the FUT7 gene into RL95-2 cells by transient transfection, and found that FUT7 up-regulation in the transfected cells was strongly associated with the augmentation of sLeX compared with the control (unpublished data). The introduction of antisense cDNA of FUT7 gene into the human lymphoid cell lines selectively down-regulated FUT7, and suppressed sLeX expression and E-selectin mediated binding (*32*). Transfection of FUT7 antisense sequence impairs the tumor proliferation (*33*). The tumor metastasis was also attenuated because of the reduction of L-selectin ligands in FUT7-deficient mice (*6*). The suppression of FUT7 expression and the determination of the effect of this inhibition on embryo implantation has not been performed.

Antisense oligodeoxynucleotides are small segments of single-strand DNA complementary to the mRNA regions of a target gene. They can interfere with translation by binding to the target mRNA, inducing degradation of the mRNA, and thereby inhibiting translation (*34, 35*). Oligodeoxynucleotides are relatively easier access to specific cellular targets and have fewer side effects. Therefore, oligodeoxynucleotides are useful in the research and practical application. In this work, we observed the effect of FUT7 suppression by introducing an antisense oligodeoxynucleotide of FUT7 gene to the cultured RL95-2 cells. We found that the expression of both FUT7 enzyme and sLeX antigen were decreased in a dose dependent manner, and the adhesion of JAR embryonic cells to RL95-2 epithelial cells was significantly reduced, compared with the untransfected control and sense oligodeoxynucleotide transfected cells. The decrease in the percent adhesion was also studied in the RL95-2 cells after single anti-sLeX antibody incubation or combined antisense oligodeoxynucleotide transfection and anti-sLeX antibody incubation. Although the combined treatment of antisense oligodeoxynucleotide transfection and anti-sLeX antibody incubation caused the strongest inhibition of adhesion, it had only a moderate effect, indicating that other ligands of L-selectin except sLeX, for example, MECA-79 epitope or heparin-like molecules, can also interact with L-selectin (*36, 37*). Further studies are needed.

In conclusion, the antisense oligodeoxynucleotide of FUT7 can significantly suppress the gene and protein expression of FUT7, and decrease sLeX synthesis in uterine epithelial cells, and thereby interrupt sLeX antigen mediated adhesion of embryonic cells to uterine epithelial cells. FUT7 may be a potential target to control embryo adhesion and implantation.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China Research grants (30270329, 30670465) and Liaoning Provincial Core Laboratory of Glycobiology and Glycoengineering grant.

REFERENCES

1. Achache, H. and Revel, A. (2006) Endometrial receptivity markers, the journey to successful embryo implantation. *Hum. Reprod. Update* **12**, 731–746.
2. Carson, D. D. (2002) The glycobiology of implantation. *Front. Biosci* **17**, 1535–1544.
3. Zhu, Z. M. and Wang, X. Q. (1998) Role for cell surface oligosaccharide in cell–cell recognition during implantation. *Mol. Hum. Reprod* **4**, 735–738.
4. Li, H., Kong, Y., and Yan, B. (2002) Regulation by ovarian hormones of alpha 1,3-fucosyltransferase gene (FUT9) expression in human endometrium. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **34**, 775–779.
5. Carson, D. D., Julian, J., Lessey, B. A., Prakobphol, A., and Fisher, S. J. (2006) MUC1 is a scaffold for selectin ligands in the human uterus. *Front. Biosci.* **111**, 2903–2908.
6. Rosen, S. D. (2004) Ligands for L-selectin: homing, inflammation, and beyond. *Annu. Rev. Immunol* **22**, 129–156.
7. Läubli, H., Stevenson, J. L., Varki, A., Varki, N. M., and Borsig, L. (2006) L-selectin facilitation of metastasis involves temporal induction of Fut7-dependent ligands at sites of tumor cell arrest. *Cancer. Res.* **66**, 1536–1542.

8. Dominguez, F., Yáñez-Mó, M., Sanchez-Madrid, F., and Simón, C. (2005) Embryonic implantation and leukocyte transendothelial migration: different processes with similar players? *J. FASEB* **19**, 1056–1060.
9. Shamonki, M. I., Kligman, I., Shamonki, J. M., Schattman, G. L., Hyjek, E., Spandorfer, S. D., Zaninovic, N., and Rosenwaks, Z. (2006) Immunohistochemical expression of endometrial L-selectin ligand is higher in donor egg recipients with embryonic implantation. *Fertil. Steril*. **86**, 1365–1375.
10. Genbacev, O. D., Prakobphol, A., Foulk, R. A., Krtolica, A. R., Ilic, D., Singer, M. S., Yang, Z. Q., Kiessling, L. L., Rosen, S. D., and Fisher, S. J. (2003) Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. *Science* **299**, 405–408.
11. Taniguchi, N., Honke, K., and Fukuda, M. (2002) Handbook of glycosyltransferases and related genes. In *Fucosyltransferases* (Oriol R, Mollicone R, Narimatsu H, eds.). pp. 205–231, Springer-Verlag, Tokyo.
12. Ma, B., Simala-Grant, J. L., and Taylor, D. E. (2006) Fucosylation in prokaryotes and eukaryotes. *Glycobiology* **16**, 158–184.
13. Escrevente, C., Machado, E., Brito, C., Reis, C. A., Stoeck, A., Runz, S., Marmé, A., Altevogt, P., and Costa, J. (2006) Different expression levels of alpha3/4 fucosyltransferases and Lewis determinants in ovarian carcinoma tissues and cell lines. *Int. J. Oncol*. **29**, 557–566.
14. Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanai, N., and Nishi, T. (1994). Expression cloning of a novel alpha 1,3-fucosyltransferase that is involved in biosynthesis of the sialyl Lewis x carbohydrate determinants in leukocytes. *J. Biol. Chem.* **269**, 14730–14737.
15. Smith, P. L., Gersten, K. M., Petryniak, B., Kelly, R. J., Rogers, C., Natsuka, Y., and Alford, J. A. (1996) Expression of the alpha(1,3)fucosyltransferase Fuc-TVII in lymphoid aggregate high endothelial venules correlates with expression of L-selectin ligands. *J. Biol. Chem.* **271**, 8250–8259.
16. Hiraiwa, N., Dohi, T., Kawakami-Kimura, N., Yumen, M., Ohmori, K., Maeda, M., and Kannagi, R. (1996) Suppression of sialyl Lewis X expression and E-selectin-mediated cell adhesion in cultured human lymphoid cells by transfection of antisense cDNA of an alpha1→3 fucosyltransferase (Fuc-T VII). *J. Biol. Chem.* **271**, 31556–31561.
17. John, N. J., Linke, M., and Denker, H.-W. (1993) Retinoic acid decreases attachment of JAR choriocarcinoma spheroids to a human endometrial cell monolayer. *Placenta* **14**, 13–24.
18. Thie, M., Röspel, R., Dettmann, W., Benoit, M., Ludwig, M., Gaub, H. E., and Denker, H. W. (1998) Interaction between trophoblast and uterine epithelium: monitoring of adhesion forces. *Hum. Reprod.* **13**, 3211–3219.
19. John, N. J., Linke, M., and Denker, H.-W. (1993) Quantitation of human choriocarcinoma spheroid attachment to uterine epithelial cell monolayers. *In Vitro Cell. Dev. Biol.* **29A**, 461–468.
20. Paria, B. C., Song, H., and Dey, S. K. (2001) Implantation: molecular basis of embryo-uterine dialogue. *Int. J. Dev. Biol.* **45**, 597–605.
21. Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., and Wang, H. (2004) Molecular cues to implantation. *Endocr. Rev.* **25**, 341–373.
22. Wang, X. Q., Zhu, Z. M., Fenderson, B. A., Zeng, G. Q., Cao, Y. J., and Jiang, G. T. (1998) Effects of monoclonal antibody directed to LeY on implantation in the mouse. *Mol. Hum. Reprod.* **4**, 295–300.
23. Lai, T. H., Shih, Ie.M., Vlahos, N., Ho, C. L., Wallach, E., and Zhao, Y. (2005) Differential expression of L-selectin ligand in the endometrium during the menstrual cycle. *Fertil. Steril.* **83**, 1297–1302.
24. Prakobphol, A., Genbacev, O., Gormley, M., Kapidzic, M., and Fisher, S. J. (2006) A role for the L-selectin adhesion system in mediating cytotrophoblast emigration from the placenta. *Dev. Biol.* **298**, 107–117.
25. Wang, H., Ge, C. H., Kong, Y., Xin, Y., and Zhu, Z. M. (2001) Ovary hormonal control of Le(y) oligosaccharide expression during peri-implantation of mouse endometrium. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **33**, 542–546.
26. Kaneko, M., Kudo, T., Iwasaki, H., Ikehara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shiina, T., Inoko, H., Saitou, N., and Narimatsu, H. (1999) Alpha1,3-fucosyltransferase IX (Fuc-TIX) is very highly conserved between human and mouse; molecular cloning, characterization and tissue distribution of human Fuc-TIX. *FEBS Lett.* **452**, 237–242.
27. Toivonen, S., Nishihara, S., Narimatsu, H., Renkonen, O., and Renkonen, R. (2002) Fuc-TIX: a versatile alpha1,3-fucosyltransferase with a distinct acceptor- and site-specificity profile. *Glycobiology* **12**, 361–368.
28. Liu, F., Qi, H. L., Zhang, Y., and Chen, H. L. (2001) Transfection of the c- erbB2/ neu gene upregulates the expression of sialyl Lewis X, a1,3-fucosyltransferase VII, and metastatic potential in a human hepatocarcinoma cell line. *Eur. J. Biochem.* **268**, 3501–3512.
29. Schuldes, H., Schleicher, D., and Mayer, G. (2003) Binding of gastrointestinal tumor cells to endothelial E- and P-selectin adhesion receptors leads to transient down-regulation of sLeX ligands in vitro. *Int. J. Colorectal. Dis.* **18**, 292–299.
30. Mas, E., Pasqualini, E., Caillol, N., El Battari, A., Crotte, C., Lombardo, D., and Sadoulet, M. O. (1998) Fucosyltransferase activities in human pancreatic tissue: comparative study between cancer tissues and established tumoral cell lines. *Glycobiology* **8**, 605–613.
31. Wagers, A. J., Lowe, J. B., and Kansas, G. S. (1996) An important role for the alpha 1,3 fucosyltransferase, FucT-VII, in leukocyte adhesion to E-selectin. *Blood* **88**, 2125–2132.
32. Hiraiwa, N., Dohi, T., Kawakami-Kimura, N., Yumen, M., Ohmori, K., Maeda, M., and Kannagi, R. (1996) Suppression of sialyl Lewis X expression and E-selectin-mediated cell adhesion in cultured human lymphoid cells by transfection of antisense cDNA of an alpha1→3 fucosyltransferase (Fuc-T VII). *J. Biol. Chem.* **271**, 31556–31561.
33. Hiller, K. M., Mayben, J. P., Bendt, K. M., Manousos, G. A., Senger, K., Cameron, H. S., and Weston, B. W. (2000) Transfection of alpha(1,3)fucosyltransferase antisense sequences impairs the proliferative and tumorigenic ability of human colon carcinoma cells. *Mol. Carcinog.* **27**, 280–288.
34. So, A., Rocchi, P., and Gleave, M. (2005) Antisense oligonucleotide therapy in the management of bladder cancer. *Curr. Opin. Urol.* **15**, 320–327.
35. Chan, J. H., Lim, S., and Wong, W. S. (2006) Antisense oligonucleotides: from design to therapeutic application. *Clin. Exp. Pharmacol. Physiol.* **33**, 533–340.
36. Toppila, S., Paavonen, T., Laitinen, A., Laitinen, L. A., and Renkonen, R. (2000) Endothelial sulfated sialyl Lewis x glycans, putative L-selectin ligands, are preferentially expressed in bronchial asthma but not in other chronic inflammatory lung diseases. *J. Respir. Cell. Mo.l Biol.* **23**, 492–498.
37. Celie, J. W., Keuning, E. D., Beelen, R. H., Dräger, A. M., Zweegman, S., Kessler, F. L., Soininen, R, and van den Born, J. (2005) Identification of L-selectin binding heparan sulfates attached to collagen type XVIII. *J. Biol. Chem.* **280**, 26965–26973.