Placenta 75 (2019) 45–53

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

FUT8 drives the proliferation and invasion of trophoblastic cells *via* IGF-1/IGF-1R signaling pathway

Ming Yu[a,1], Xinyuan Cui[a,1], Hao Wang[a], Jianwei Liu[a], Huamin Qin[b], Shuai Liu[a,**], Qiu Yan[a,*]

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

[b] Department of Pathology, The Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, 116011, China

ARTICLE INFO

Keywords:
Implantation
Trophoblasts
FUT8
IGF-1R

ABSTRACT

Introduction: Trophoblast proliferation and invasion are essential for embryo implantation and placentation. Protein glycosylation is one of the most common and vital post-translational modifications, regulates protein physical and biochemical properties. FUT8 is the only known fucosyltransferase responsible for catalyzing α1,6-fucosylation in mammals, and α1,6-fucosylated glycoproteins are found to participate in various physiopathological processes. However, whether FUT8/α1,6-fucosylation modulates the functions of trophoblastic cells remains elusive.

Methods: FUT8 in human placenta villi during 6-8 gestational weeks and trophoblastic cells were detected by Western blot and immunofluorescent staining. α1,6-fucosylation in tissues or cells were measured by Lectin LCA (*Lens culinaris*) fluorescent staining and Lectin blot. FUT8 expression was down-regulated by siRNA transfection in JAR and JEG-3 cells, and cell viability, motility and invasiveness ability were detected by the functional experiments. α1,6-fucosylation of insulin-like growth factor receptor (IGF-1R) was examined by immunoprecipitation, and the amount of phosphorylated IGF-1R was detected in FUT8 down-regulated JAR cells.

Results: Human placenta villi and trophoblastic cells expressed FUT8/α1,6-fucosylation. Knockdown FUT8 by siRNA transfection suppressed the proliferation, epithelial-mesenchymal transition, migration and invasion of JAR and JEG-3 cells. Furthermore, we found that FUT8 modified the α1,6-fucosylation of IGF-1R, and regulated IGF-1 dependent activation of IGF-1R, MAPK and PI3K/Akt signaling pathways in JAR cells.

Conclusions: Our results implicate a critical role for FUT8 in maintaining the normal functions of trophoblastic cells, suggesting manipulating FUT8 may be an effective approach in pregnancy.

1. Introduction

Embryo development, embryo implantation and placental development are prerequisites for successful pregnancy in mammals. Trophoblasts are specialized cells of the placental villi, play vital roles in regulating these complex biological events [1,2]. Trophoblastic cells have highly proliferative and invasive properties [3], and insufficient trophoblast proliferation and invasion are considered as one of the crucial etiological factors for certain pregnancy-associated diseases, such as early spontaneous miscarriage (ESM), preeclampsia (PE), fetal intrauterine growth restriction (FGR) and even infertility [4–6].

During early pregnancy (the first trimester), the extravillous trophoblasts (EVTs) undergo epithelial-mesenchymal transition (EMT), in which epithelial cells lose the tight junction (reduced E-cadherin expression), change into a mesenchymal phenotype (enhanced Vimentin and N-cadherin expression) and as a consequence, gain a highly motility [7]. In addition, several extracellular matrix (ECM) degradation-related proteins (MMP-2 and MMP-9) are expressed by the EVTs to maintain the invasive properties [8]. The EVTs invade to the endometrial decidua cells and the muscular layer of uterine spiral arteries to provide fetoplacental perfusion and establish the maternal-fetal blood circulation [9]. Inadequate EVTs migration or invasion leads to

Abbreviations: DAPI, 2-(4-amidinophenyl)-6-indolecarbamidine; EVTs, extravillous trophoblasts; EMT, epithelial-mesenchymal transition; FUT8, α1,6-fucosyltransferase VIII; IGF-1, Insulin-like growth factor 1; IGF-1R, Insulin-like growth factor 1 receptor; LCA, Lens culinaris; MAPK, Mitogen-activated protein kinase; MMP-2, Matrix metalloprotein 2; MMP-9, Matrix metalloprotein 9; PCNA, Proliferating cell nuclear antigen; PI3K, Phosphoinositide 3-kinase

* Corresponding author.
** Corresponding author.
E-mail addresses: liushuai_129@163.com (S. Liu), yanqiu63@126.com (Q. Yan).

[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.placenta.2018.11.005
Received 14 June 2018; Received in revised form 7 November 2018; Accepted 15 November 2018

shallow interstitial invasion and unconverted narrow spiral arteries, which in turn causes implantation failure and placental dysfunction [10].

Protein glycosylation is one of the most common and important post-translational modifications, closely correlates with numerous psychological and pathological progresses including reproduction, immune response and tumorigenesis [11–13]. According to the glycosylation sites of the polypeptides, glycosylation is classified into two major forms: N-glycosylation (sequon: Asn-X-Ser/Thr, X represents any amino acid except Pro) and O-glycosylation (sequon: Ser/Thr) [14]. Fucosylation is an important subtype of glycosylation, in which a fucose is transferred from GDP-fucose to the N-glycans, O-glycans and lipid-linked glycans by 11 fucosyltransferases (FUT1-11) to form α1,2-, α1,3/4-, or α1,6-linkage. Alternatively, fucose can be directly linked to the Ser/Thr residues within the epidermal growth factor-like or thrombospondin repeats sequences by 2 specific protein O-linked fucosyltransferases (poFUT1-2) [15]. In mammals, FUT8 is the only fucosyltransferase responsible for the α1,6-fucosylation by adding fucose to the innermost GlcNAc of N-glycans carried by the glycoproteins. There is an extensive literature suggests that certain glycoproteins such as TGF-β receptor, EGF receptor, and PDGF receptor are α1,6-fucosylated, and this specific modification is found to play a crucial role in mediating the homologous ligands binding ability and ligands-activated downstream signaling pathways [16,17]. Meanwhile, FUT8 knockout mice exhibited severe growth retardation, early death during postnatal development, emphysema-like changes in the lung and a schizophrenia-like phenotype, suggesting FUT8/α1,6-fucosylatioin is essential for maintaining the normal physiological homeostasis [18,19]. We have previously found that poFUT1 significantly promotes proliferation, migration and invasion of the trophoblastic cells *in vitro* [20,21]. However, the role of FUT8/α1,6-fucosylation in modulating the proliferative and invasive behaviors of trophoblastic cells is largely unknown.

The function of trophoblasts is precisely controlled by numerous factors including hormones, growth factors, cytokines and their relative receptors during implantation and placentation [22]. Insulin-like growth facor-1 (IGF-1) is a secreted protein, found to be maternally produced in the human uterine fluid during the pre-implantation period [23]. In rat, IGF-1 is strongly expressed in the uterine endometrial epithelium and also presented in the endometrial basal lamina at the implantation phase [24]. Despite IGF-1 is not generated by trophoblastic cells, accumulating evidence showed that IGF-1 plays a vital role in stimulating the proliferation, migration and invasion of trophoblastic cells in a paracrine manner [25–27]. IGF-1 receptor (IGF-1R) is a transmembrane glycoprotein expressed on cell surface, and it consists two α subunits located extracellularly and two β subunits span the membrane [28]. In response to IGF-1 binding, α subunits induce the tyrosine autophosphorylation of the β subunits, and activates several intracellular signaling pathways (MAPK, PI3K/Akt), by which to promote cell survival, viability and motility [29]. It has been reported that statins (pravastatin or cerivastatin) and N-glycosylation inhibitors attenuated IGF-induced trophoblast proliferation by restraining the whole N-glycans of IGF-1R in first trimester villous tissue explants [30]. However, whether α1,6-fucosylated N-glycans of IGF-1R influences the IGF-1/IGF-1R signaling pathway activation, thus regulates the proliferation and invasion of trophoblasts remains undefined.

In this study, we found that human placenta villi and trophoblastic cells expressed abundant FUT8 and α1,6-fucosylation. Knockdown FUT8 by siRNA transfection impaired the proliferation, migration and invasion of JAR and JEG-3 cells. More importantly, FUT8 modified the α1,6-fucosylation of IGF-1R, and regulated IGF-1 dependent activation of IGF-1R, MAPK and PI3K/Akt signaling pathways in JAR cells. Taken together, our study clearly demonstrated that FUT8/α1,6-fucosylation drives the competent trophoblastic cells and manipulating FUT8 may be an effective approach to regulate embryo implantation and placentation.

2. Materials and methods

2.1. Human placental villi tissue collection

All experimental protocols for human study were in accordance with the approved guidelines by the Institutional Review Boards of Dalian Medical University. Written informed consent was obtained from all subjects prior to sample collection. Tissues were obtained from the Secondary Affiliated Hospital of Dalian Medical University from 2017 to 2018. The pregnant women at the ages of 25–35 were confirmed by ultrasound detection at 6–8 gestational weeks. Human placenta villi from non-drug abortion women were used for immunohistochemical fluorescent staining and protein extraction.

2.2. Cell culture

JAR and JEG-3 cell lines were obtained from the American Type Culture Collection. The cells were maintained in DMEM/F12 (Hyclone) conditional medium supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin. Cells were cultured in a humidified atmosphere containing 5% CO2 at 37 °C. The medium was renewed every 2–3 days.

2.3. Real-time quantitative PCR

Cells were treated with RNAiso Plus reagent (Takara) for RNA extraction, and the PrimeScript RT reagent Kit with a gDNA Eraser kit (Takara) was used to synthesize cDNA. SYBR Premix Ex Taq (Takara) was used for q-PCR. The primers were listed in Table 1.The reactions were performed using the Applied Biosystems 7500 Fast Real-time PCR System (Life Technologies, USA). Quantified data were normalized to those of GAPDH, and the relative quantity was calculated using the $2-\Delta\Delta^{CT}$ method.

Table 1
The primers used in this study.

Gene	Primers
FUT8	5′-TAT GCT TCA GCC TTG ATG-3′ (forward), 5′-TTG GTG ACT GAC AAG AT GG-3′ (reverse);
PCNA	5′-TAA AGA AGA GGA GGC GGT AA-3′ (forward), 5′-TAA GTG TCC CAT GTC AGC AA-3′(reverse);
Cyclin D1	5′-TGT CCT ACT ACC GCC TCA CA-3′ (forward), 5′-CTT GGG GTC CAT GTT CTG CT-3′ (reverse);
Cyclin E1	5′-TGC AGC CAA ACT TGA GGA AA TC-3′ (forward), 5′-TAG TCA GGG GAC TTA AAC GC CA-3′ (reverse);
Vimentin	5′-CGT CTC TGG CAC GTC TTG AC-3′ (forward), 5′-GCT TGG AAA CAT CCA CAT CGA-3′ (reverse);
N-cadherin	5′-AAA GAA CGC CAG GCC AAA C-3′ (forward), 5′-GGC ATC AGG CTC CAC AGT GT-3′ (reverse);
E-cadherin	5′-CAA CGA CCC AAC CCA AGA A-3′ (forward), 5′—CCG AAG AAA CAG CAA GAG CA-3′ (reverse);
MMP-2	5′-TGA TCT TGA CCA GAA TAC CAT CGA-3′ (forward), 5′-GGC TTG CGA GGG AAG AAG TT-3′ (reverse);
MMP-9	5′—CCT GGA GAC CTG AGA ACC AAT C-3′ (forward), 5′—CCA CCC GAG TGT AAC CAT AGC-3′ (reverse);
GAPDH	5′-GCA CCG TCA AGG CTG AGA AC-3′ (forward), 5′-TGG TGA AGA CGC CAG TGGA-3′ (reverse).

2.4. Transfection

JAR or JEG-3 cells were seeded onto six-well plates or 96-well plates. When cells reached 70% confluence, scramble or FUT8 siRNA: 5′-GUG GAG UGA UCC UGG AUA UTT-3′ (sense), 5′-AUA UCC AGG AUC ACU CCA CTT-3′ (antisense) (synthesized from GenePharma) were transiently transfected into the cells using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. The transfection reagent was removed 6 h later. Total protein and RNA was collected after 48 h.

2.5. Western and lectin blot

Equal proteins were loaded onto 10% SDS-PAGE gels, and then were transferred onto a nitrocellulose membrane (Merck Millipore). After blocking with 5% non-fat dry milk for 2 h, the membranes were incubated at 4 °C overnight with the primary antibody: FUT8 and IGF-1R (Santa Cruz); p-IGF-1R(Tyr^{1161}) (Merck Millipore); Erk1/2, p-Erk1/2, Akt and p-Akt(Tyr^{308}) (Cell Signaling Technology); CK-7, PCNA, Cyclin D1, Cyclin E1, Vimentin, N-cadherin, E-cadherin, MMP-2, MMP-9 and GAPDH (Proteintech); biotinylated LCA (Vector Laboratories). Next, the membranes were incubated with HRP-labeled goat anti-mouse IgG, HRP-labeled goat anti-rabbit IgG or HRP-labeled streptavidin for 1 h. An enhanced chemiluminescence (ECL) detection system (Bio-Rad, USA) was used to visualize immunoreactive bands.

2.6. Immunofluorescent and lectin fluorescent staining

Cover-slips (cells) or frozen slices (tissues) were fixed in 4% paraformaldehyde or cold acetone for 30 min, followed by blocking with 1% goat serum (Beyotime) for 2 h. Next, the cover-slips or slices were incubated with antibodies: FUT8 (Santa Cruz), CK-7 (Proteintech), IGF-1R (Santa Cruz) or Rhodamine labeled LCA (Vector Laboratories) at 4 °C overnight followed by incubation with the FITC or TRITC-conjugated second antibody for 1 h. After counterstaining with DAPI (blue) for 5 min, anti-fade solution (Beyotime) was added to mount the cover-slips or slices before imaging under the fluorescent microscope (Olympus, Japan).

2.7. Cell viability assay

Cells (5000/well) were seeded in 96-well plates, and transfected with scramble or FUT8 siRNA. 10 μl of the Cell Counting Kit-8 (CCK-8) reagent (Dojindo Molecular Technologies) was added to 90 μl of the FBS-free medium in each well, followed by 2 h incubation at 37 °C. Subsequently, the absorbance value (OD) was measured at 450 nm using a microplate reader (Thermo Fisher Scientific). Each assay was conducted in triplicate and repeated at three times.

2.8. Matrigel cell invasion and transwell cell migration assay

For matrigel cell invasion assay, transwell inserts (6.5 mm, Costar) containing polycarbonate filters with 8 μm pores were precoated with 50 μl of 1 mg/ml matrigel matrix (Becton Dickinson). For cell migration assay, the inserts were not precoated with matrigel. Cells in serum-free medium were plated into the upper chamber, whereas medium with 10% FBS was added to the lower chamber. After incubating for 24 h, the cells on the upper side of the inserts were removed by cotton swab. The bottom of the inserts were fixed in methanol and stained with crystal violet. The number of invaded or migrated cells was counted under a light microscope (Olympus) in five random fields. Three independent experiments were performed.

2.9. Gelatin zymography assay

The cell supernatants were collected and used as the samples to detect the MMP-2 and MMP-9 activity. The proteins were electrophoresed on 10% SDS-PAGE gels containing 1% gelatin. After electrophoresis, the gels were washed in 2.5% Triton X-100 (1 h, RT), followed by incubating in 50 mM Tris-Cl, pH 7.6, and 5 mM $CaCl_2$ (18 h, 37 °C). The gels were stained with 0.1% Coomassie blue R250 and destained in 10% methanol and 10% acetic acid in H_2O.

2.10. Immunoprecipitation

Immunoprecipitation was performed with the Dynabeads® Protein G Kit (Life technologies, USA) by following the standard procedure. Briefly, total protein lysates were added to the Dynabeads-IGF-1R antibody (Ab) complex for 30 min at RT, followed by washing 3 times. Subsequently, the Dynabeads-Ab-protein complex was mixed with elution and lysis buffer, and incubated 15 min at 70 °C to denature the proteins.

2.11. Statistical analysis

GraphPad Prism® (GraphPad Software Inc) was used for statistical analysis. All experiments were performed at least 3 independent times, and the data were presented as means ± SEM. For the analysis of difference between groups, independent-samples *t*-test or one-way ANOVA was performed, and the statistical significance was indicated as the follows, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

3. Results

3.1. Expression of FUT8 and α1,6-fucosylation in human placenta villi and trophoblastic cells

To detect the expression of FUT8 and α1,6-fucosylation in human placenta villi from the first trimester of pregnancy, we collected the tissues from woman at 6–8 weeks of gestation. Immunohistofluorescent staining and Lectin *Lens culinaris* (LCA, recognizes the α1,6-fucosylated N-glycans) fluorescent staining were performed to examine the expression of FUT8 and α1,6-fucosylation, respectively. The fluorescent staining images showed that the villi, verified by CK-7, expressed FUT8 and α1,6-fucosylation (Fig. 1 A). Human trophoblastic JAR and JEG-3 cells, which have an EVT phenotype [31], were also found to express FUT8 and α1,6-fucosylation (Fig. 1 B). Western blot and Lectin blot were conducted to further confirm the results (Fig. 1C and D).

3.2. FUT8 knockdown by siRNA transfection reduced the α1,6-fucosylation level in JAR and JEG-3 cells

To investigate the roles of FUT8/α1,6-fucosylation in trophoblastic cells, JAR and JEG-3 cells were transiently transfected with FUT8 siRNA, and down-regulated FUT8 expression were confirmed by Q-PCR (Fig. 2A and B) and Western blot (Fig. 2C and D). Meanwhile, Lectin LCA fluorescent staining (Fig. 2E and F) and Lectin blot (Fig. 2G and H) showed that FUT8 knockdown markedly reduced the α1,6-fucosylation in JAR and JEG-3 cells.

3.3. FUT8 modulated the proliferation, migration and invasion of JAR and JEG-3 cells

We first detected the effects of FUT8 down-regulation on cell viability. CCK-8 assay (Fig. 3A), EdU incorporation assay (Supplementary Fig. A) data showed that FUT8 knockdown suppressed the cell proliferation. The mRNA and protein expression of PCNA (marker of cell proliferation) (Supplementary Fig. B), Cyclin D1 (marker of the early G1 phase) and Cyclin E1 (function at the G1/S transition) were also decreased in FUT8 knockdown cells (Fig. 3B and C).

In addition, Q-PCR and Western blot data showed that FUT8 down-regulation was along with an enhancement of E-cadherin expression,

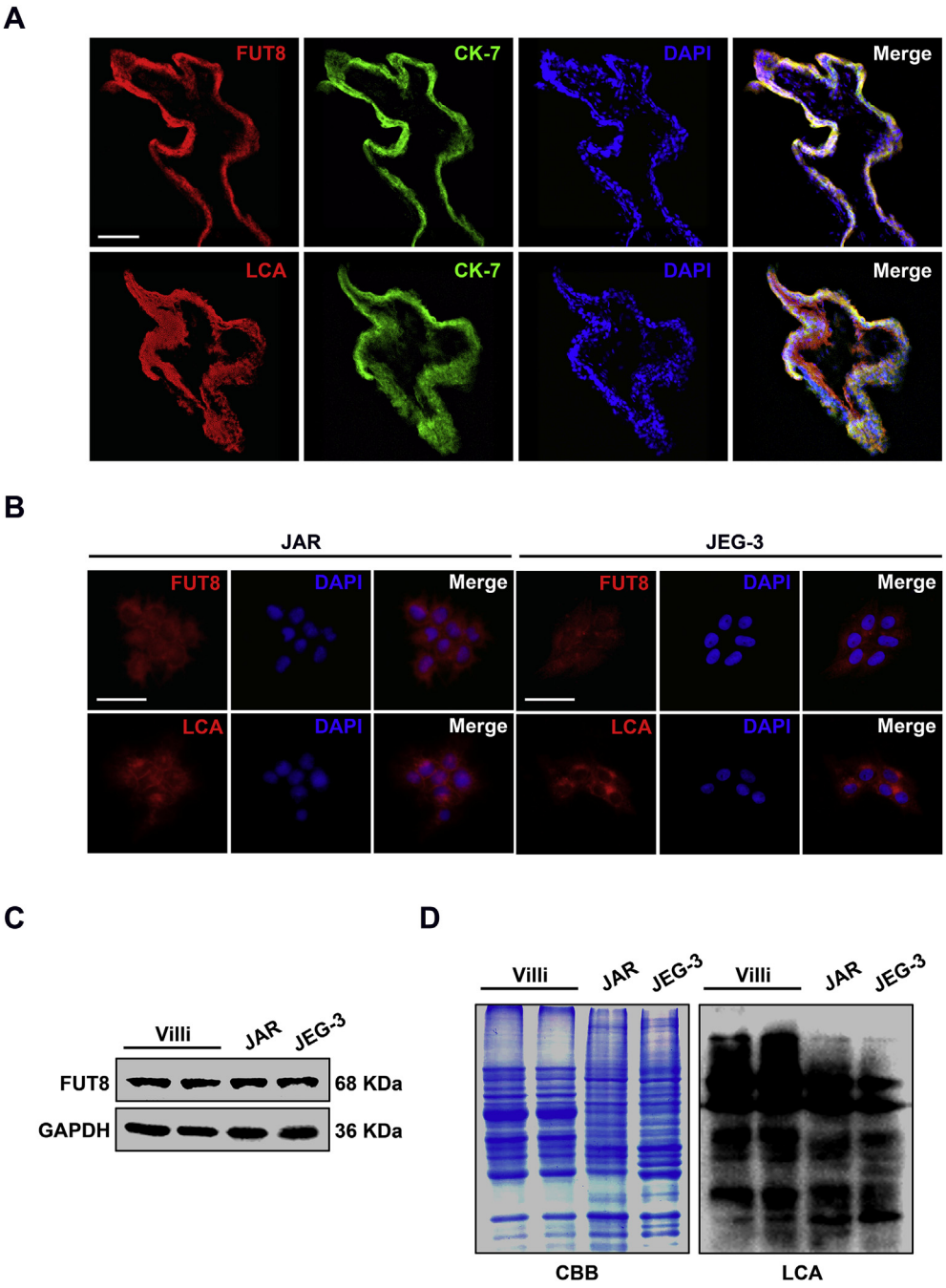

Fig. 1. Expression of FUT8 and α1,6-fucosylation in human placenta villi and trophoblastic cells. Representative fluorescent staining of FUT8 and α1,6-fucosylation in human placenta villi **(A)** as well as in JAR and JEG-3 cells **(B)**. FUT8 expression and α1,6-fucosylation in tissues and cells were also detected by Western blot **(C)** and LCA Lectin blot **(D)**. CK-7 (green) was stained as the villi marker. Nuclei were stained with the DAPI (blue). Bars represent 100 μm in (A), and 50 μm in (B). GAPDH was used as an internal control for Western blot. Coomassie brilliant blue (CBB) staining showed the equal amounts of protein loading in each lane for Lectin blot.

whereas a reduction of Vimentin and N-cadherin expression, indicating that FUT8 knockdown suppressed the EMT (Fig. 3D and E). Transwell migration assays were performed to further confirm that FUT8 knockdown markedly inhibited the cell migration (Fig. 3I). Q-PCR, Western blot and gelatin zymograogy data also showed that FUT8 knockdown resulted in a reduction of MMP-2 and MMP-9 (involved in the breakdown of extracellular matrix) expression and activity (Fig. 3F-H). Matrigel cell invasion assays were performed to further verify that FUT8 down-regulation impaired the invasiveness capacity of JAR and JEG-3 cells (Fig. 3J). To verify the similar functions of FUT8 on normal human trophoblasts, placental villous explants were performed. The outgrowth length of EVT migration was measured at 72 h after FUT8 siRNA tranfection in the explants. As shown in Supplementary Fig. C, the outgrowth distance in the FUT8 knockdown group displayed a decrease compared with that of the control group. Meanwhile, Q-PCR results confirmed that FUT8 down-regulation was associated with a decreased mRNA expression of PCNA, N-cadeherin, Vimentin, MMP-2 and MMP-9 in the explanted trophoblastic cells (Supplementary Fig. D). These results suggest that FUT8 plays a pivotal role in modulating the functions of trophoblastic cells.

3.4. FUT8 modified α1,6-fucosylated IGF-1R and regulated the IGF-1/IGF-1R signaling pathway

To determine whether IGF-1R is α1,6-fucosylated and α1,6-fucosylation influences the IGF-1R activation in JAR cells, IGF-1R was immunoprecipitated. As shown in Fig. 4A (the top row, lane 1), Western blot results clearly showed that IGF-1R was recognized by Lectin LCA, which suggested the α1,6-fucosylation of IGF-1R. As expected, the band of α1,6-fucosylated IGF-1R was decreased in FUT8 knockdown cells (Fig. 4A the top row, lane 2). It is also noteworthy that FUT8 knockdown markedly suppressed the activation of IGF-1R as compared with

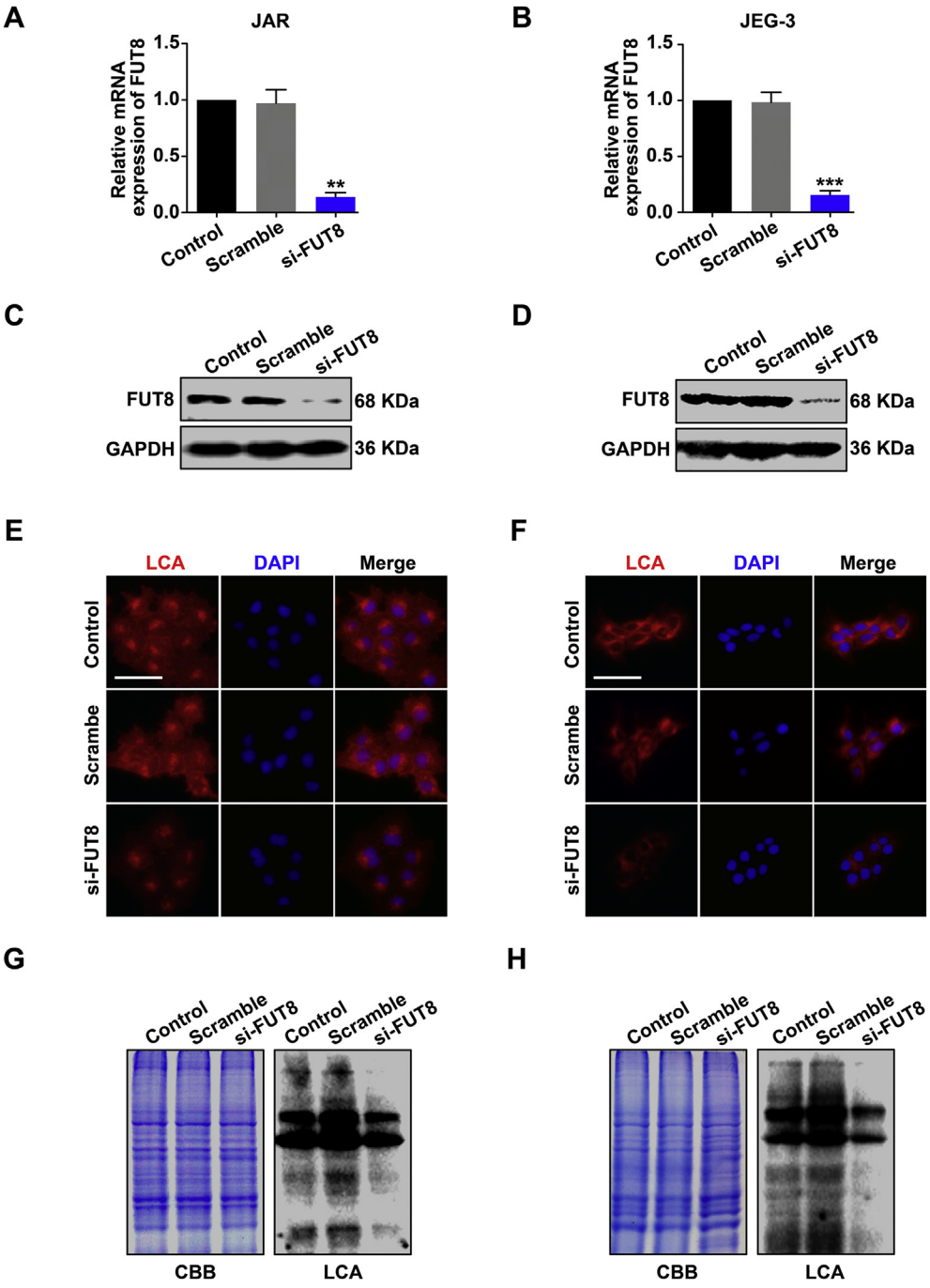

Fig. 2. FUT8 knockdown by siRNA transfection reduced the level of α1,6-fucosylation in JAR and JEG-3 cells. JAR and JEG-3 cells were transiently transfected with Scramble siRNA (negative control) and FUT8 siRNA for 48 h. FUT8 expression were detected by Q-PCR **(A, B)** and Western blot **(C, D)** in no-treated control, Scramble control and FUT8 knockdown cells. The α1,6-fucosylation level was examined by Lectin fluorescent staining **(E, F)** and Lectin blot **(G, H)**. Nuclei were stained with the DAPI (blue). Bars represent 50 μm. GAPDH was used as an internal control for Western blot. CBB staining showed the equal amounts of protein in each lane for Lectin blot. Data are presented as mean ± SD from three independent experiments, *$p < 0.05$.

the scramble control (Fig. 4A the moddle row). In addition, results showed that the total IGF-1R expression (Fig. 4A the bottom row) and its subcellular localization (Fig. 4B) were not influenced in FUT8 down-regulated cells.

To assess the effects of α1,6-fucosylation on IGF-1R activation, transfected cells were treated without or with IGF-1 (50 ng/ml) for 15 min, and Western blot results displayed that knockdown FUT8 significantly reduced the level of p-IGF-1R (Tyr^{1161}), p-Erk1/2 and p-Akt (Tyr^{308}), both basally and post IGF-1 treatment (Fig. 4C). IGF-1/IGF-1R-activated MAPK and PI3K/Akt signaling pathways was found to play a critical role in regulating the proliferation and invasion of trophoblastic cells. Consistently, the data herein demonstrated that AG1024 (inhibitor of IGF-1R autophosphorylation) treatment significantly decreased the amount of p-IGF-1R (Tyr^{1161}), p-Erk1/2 and p-Akt (Tyr^{308}) as well as the expression of relevant biomarkers when compared with the vehicle control in JAR cells (Fig. 4D).

Next, we asked whether FUT8 knockdown attenuated IGF-1 induced signaling pathways activation and the downstream target biomarkers expression. Cells were treated with FUT8 siRNA or AG1024 (5 μM) for 24 h, followed by stimulating with the IGF-1 (50 ng/ml) for 48 h. Western blot results showed that IGF-1 treatment led to an increase in the p-IGF-1R (Tyr^{1161}), p-Erk1/2 and p-Akt (Tyr^{308}) level, coincident with an up-regulation in the PCNA, Cyclin D1, Vimentin and MMP-2 level, whereas a down-regulation in the E-cadherin level when compared with the control (Fig. 4E lane 2 versus lane 1). However, both FUT8 siRNA and AG1024 treatment attenuated IGF-1 induced IGF-1R, Erk1/2 and Akt activation as well as alleviated IGF-1 mediated PCNA, Cyclin D1, Vimentin and MMP-2 expression. In contrast, E-cadherin expression in IGF-1 + FUT8 siRNA or IGF-1 + AG1024 treated cells was enhanced as compared with the IGF-1 treated cells (Fig. 4E lane 3 and

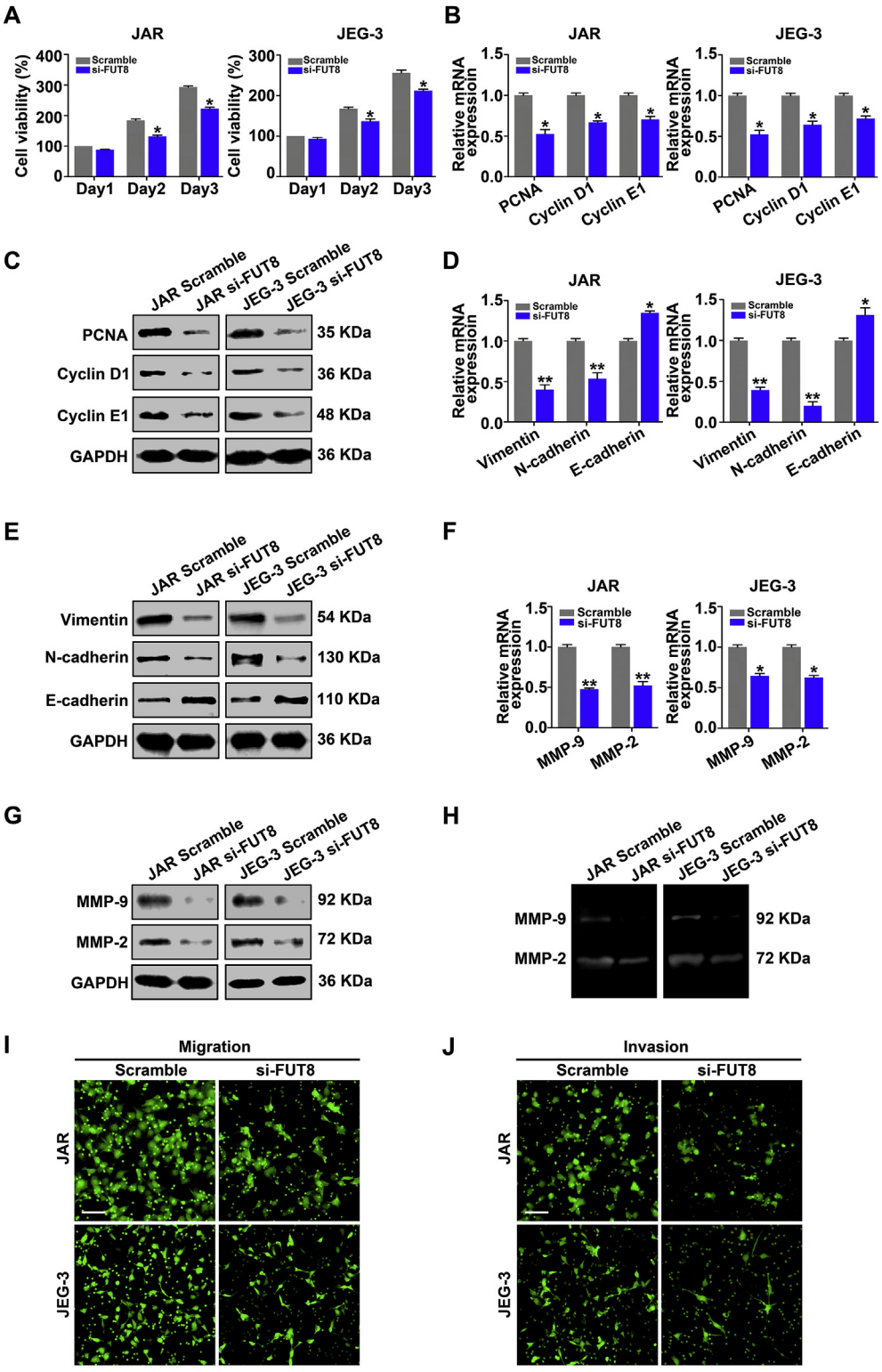

Fig. 3. FUT8 modulated the proliferation, migration and invasion of JAR and JEG-3 cells. JAR and JEG-3 cells were transiently transfected with Scramble siRNA and FUT8 siRNA. Cell viability was analyzed by CCK-8 assays at day 1, day 2 and day 3 **(A)**. mRNA and protein level of PCNA, Cyclin D1 and Cyclin E1 were detected by Q-PCR **(B)** and Western blot **(C)**. mRNA and protein level of Vimentin, N-cadherin and E-cadherin were detected by Q-PCR **(D)** and Western blot **(E)**. mRNA and protein level of MMP-2 and MMP-9 were detected by Q-PCR **(F)** and Western blot **(G)**. MMP-2 and MMP-9 activity were tested by gelatin zymography assays **(H)**. Cell motility and invasiveness ability were examined by transwell migration **(I)** and invasion assays **(J)**. Bars represent 50 μm. GAPDH was used as an internal control for Western blot. Data are presented as mean ± SD from three independent experiments, *$p < 0.05$; **$p < 0.005$.

4). These data obtained above indicates that α1,6-fucosylation of IGF-1R plays a critical role in modulating the IGF-1/IGF-1R signaling transduction, thereby regulating the biological functions of trophoblastic cells.

4. Discussion

Glycans on cell surface affect multiple reproductive processes including sperm-egg binding, embryo development and endometrium-embryo interactions [32–34]. Glycosylation is a biochemical and enzymatic reaction by which a monosaccharide is added to the acceptor substrate through the specific glycosyltransferase, and over 200 glycosyltransferase genes have been verified to date [35]. Extensive research has been carried out to clarify the roles of certain glycosyltransferases and their corresponding glycans carried by specific glycoprotines in mediating the biological behavior of the trophoblasts. Huang, M. C. and Shyu, M. K. et al. found two kinds of O-glycosyltransferases: polypeptide acetylgalactosaminetransferase-II (GALNT2)

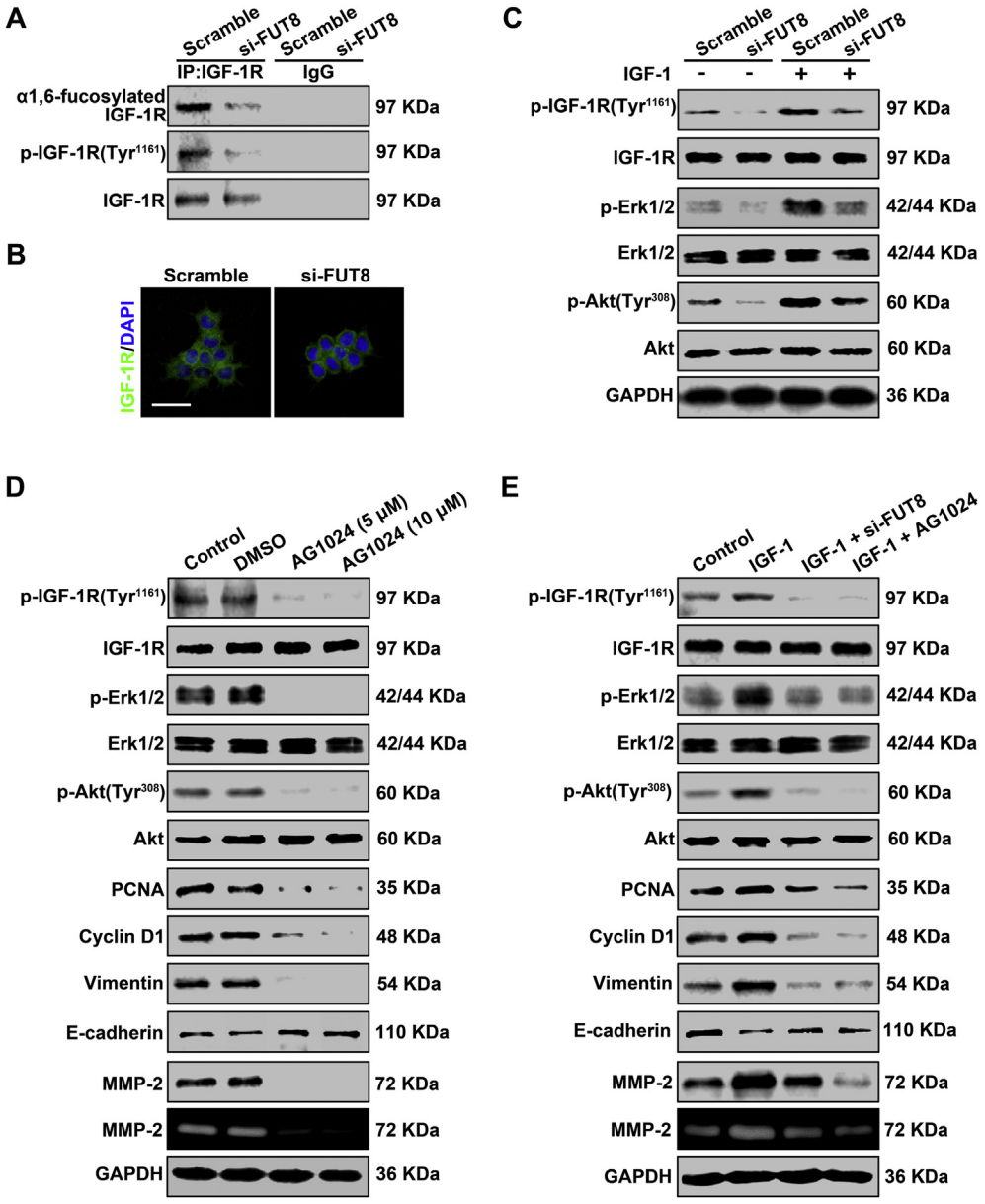

Fig. 4. FUT8 modified α1,6-fucosylation of IGF-1R and regulated the IGF-1/IGF-1R signaling pathway. JAR cells were transiently transfected with Scramble siRNA and FUT8 siRNA, and the total protein lysates were immunoprecipitated with IGF-1R antibodies and IgG (Negative control). The samples were immunoblotted to detect the amount of α1,6-fucosylated IGF-1R, p-IGF-1R (Tyr^{1161}) and total IGF-1R **(A)**. Representative fluorescent staining of IGF-1R in Scramble siRNA and FUT8 siRNA treated cells **(B)**. The expression of p-IGF-1R (Tyr^{1161}), IGF-1R, p-Erk1/2, Erk1/2, p-Akt (Tyr^{308}) and Akt in JAR cells after different treatments as mentioned were detected by Western blot **(C)**. The expression of p-IGF-1R (Tyr^{1161}), IGF-1R, p-Erk1/2, Erk1/2, p-Akt (Tyr^{308}), Akt, PCNA, Cyclin D1, Vimentin, E-cadherin and MMP-2 in JAR cells after different treatments as mentioned were detected by Western blot **(D, E)**. MMP-2 activity was tested by gelatin zymography assays **(D)** and **(E)**. Nuclei were stained with the DAPI (blue). Bar represents 50 μm. GAPDH was used as an internal control for Western blot. Data are presented as mean ± SD from three independent experiments.

and β1,4-galactosyltransferase-III (B4GALT3) suppressed the invasion but enhanced the adhesion of EVT cells to extracellular matrix through modified the O-glycans on integrin β1 in the late stages of pregnancy [36,37]. Eiko Yamamoto and colleagues' study discovered that knockdown the expression of N-acetylglucosaminyltransferase V (GnT-V) enhanced the migratory and invasive ability of EVT cells via diminishing the β1,6-GlcNAc branching on integrin α5β1 [38]. It is also noteworthy that aberrant expressions of certain glycosyltransferases in trophoblasts might be closely associated with early spontaneous abortion (ESM). For instance, placental villi of ESM exhibited decreased GnT-V and its product, β1,6-GlcNAc branching, together with elevated GnT-III and its product, bis-GlcNAc branching, on integrin β1 relative to the normal placental villi [39]. Meanwhile, our previous study showed that poFUT1 expression was down-regulated in placental villi from abortion patients, and poFUT1 down-regulation suppressed the proliferation and invasion of JAR cells through inactivating the MAPK and PI3K/Akt signaling pathways [21]. Prior study also found that FUT7, the key enzyme for the biosynthesis of sialy Lewis X oligosaccharide, is highly expressed in mouse blastocyst and human villi tissues. FUT7/sLeX of trophoblasts promoted the adhesion ability to the endometrial cells *in vitro* [40]. FUT8 expression silencing by siRNA transfection abrogated the cell viability, motility and invasiveness (Fig. 3A, I and J; Supplementary Fig. C), and significantly inhibited the mRNA and protein expression of the relevant biomarkers in trophoblastic cells (Fig. 3B-H; Supplementary Fig. D). Combined with others' studies, we believe that appropriate expression of certain glycosyltransferases in trophoblasts is prerequisite for maintaining the normal physiological functions of placenta.

It is well elucidated that glycosylation influences glycoprotein folding, conformation, solubility and stability [41]. Distinct modified glycans on glycoproteins also play heterogeneous roles in regulating cell adhesion, proliferation and invasion through altering the adhesion molecule distribution, receptor activation and protein interactions [42,43]. Numerous glycoproteins are found to be α1,6-fucosylated, and this modification is critical for modulating the biological functions in many physiological processes and diseases. Knockout mice deficient in FUT8 presented phenotypes of growth retardation, death during postnatal development, and lung emphysema. These severe phenotypes are possibly ascribed to the attenuation of TGF-β and EGF signaling pathways, and α1,6-fucosylation of TGF-βR and EGFR are shown to be

essential for their ligands binding and receptor activation [19,44]. In addition, aberrant α1,6-fucosylation catalyzed by FUT8 is identified to be closely associated with tumor EMT, metastasis and drug resistance. Agrawal, P. et al. revealed that FUT8 was up-regulated in metastatic melanoma, and increased α1,6-fucosylation of neural cell adhesion molecule L1 (L1CAM) impacted L1CAM supported melanoma invasion [45]. It is also reported that FUT8 was up-regulated by TGF-β, which is a well-known inducer for EMT, in breast cancer and non-small-cell lung cancer cells. Blockage α1,6-fucosylation of TGF-βR inactivated TGF-β signaling, thus restricting breast cancer cell metastasis [46]. IGF-1R is an N-glycosylated protein, which contains 16 predicted N-glycosylation sites. It is established that restrained general N-glycans of IGF-1R by statins or N-glycosylation inhibitors altered receptor presentation at the trophoblastic cell surface and attenuated IGF-1 or IGF-II induced cell proliferation [30]. Results herein provide evidence that IGF-1R is α1,6-fucosylated, and down-regulated the FUT8 expression decreased the α1,6-fucosylation of IGF-1R but not the total protein expression or localization of IGF-1R in JAR cells (Fig. 4A and B). We further investigated that decreased α1,6-fucosylated IGF-1R was accompanied with reduced p-IGF-1R(Tyr^{1161}) expression, both basally and post IGF-1 treatment (Fig. 4A and C). Our data is supported also by a study which revealed that elevated FUT8 expression and activity could affect the aging process by altering the IGF-1/IGF-1R signaling pathway in mouse liver cells [47]. Collectively, these findings highlight that α1,6-fucosylation of IGF-1R is required for the IGF-1R activation in trophoblastic cells.

IGF-1/IGF-1R axis plays a fundamental role in regulating many physiological events including embryo implantation, decidualization and fetal growth [48–50]. Meanwhile, accumulating studies demonstrated that aberrant IGF-1/IGF-1R axis, or in other word, insufficient IGF-1R activation leads to endometrial dysfunction, preeclampsia and even infertility. For instance, IGF-1 knockout female mice were reported to infertile, and exhibited uterine hypoplasia, suggesting that IGF-1 was crucial for uterine viability and receptivity [51]. It was also identified that heterozygous IGF-1R mutations within the intracellular kinase domain were closely associated with the intrauterine and postnatal growth failure [52,53]. MAPK and PI3K/Akt signaling pathways are evolutionarily conserved networks that responsible for cell mitosis, adhesion, EMT, migration and invasion by transmitting signals from many kinds of growth factors including IGF-1. As reported, IGF-1 facilitated the attachment of mouse blastocyst to human endometrial Ishikawa cells by increasing the apical fibronectin on mouse blastocyst, which this phenomenon was impaired by treating with the PI3K/Akt inhibitor [54]. IGF-1 was also found to promote proliferation and migration of porcine trophectoderm cells through MAPK and PI3K/Akt signaling pathways *in vitro* [26]. Additionally, a robust literature suggests that IGF-1/IGF-1R axis plays a key role in regulating EMT via activating the MAPK and PI3K/Akt signaling pathways in breast, gastric and prostate cancer cells [55,56]. The present results clearly showed that IGF-1 prompted proliferation (enhanced PCNA and Cyclin D1 protein level), EMT (enhanced Vimentin and decreased E-cadherin protein level) and invasion (elevated MMP-2 protein level) of JAR cells (Fig. 4 E). By using the IGF-1R autophosphorylation inhibitor, AG1024, we confirmed that IGF-1R inactivation attenuated the elements of MAPK and PI3K/Akt signaling pathways, as well as the relevant biomarkers for cell proliferation, EMT and invasion, both under normal or IGF-1 treatment conditions (Fig. 4D and E). As expected, FUT8 knockdown significantly reduced the amount of IGF-1 mediated p-IGF-1R (Tyr^{1161}), p-Erk1/2 and p-Akt (Tyr^{308}), coincident with attenuating the expression of IGF-1 induced PCNA, Cyclin D1, Vimentin and MMP-2 (Fig. 4 E). These results indicate that IGF-1 robustly orchestrates the physiological functions of trophoblastic cells during pregnancy, and more importantly, α1,6-fucosylation of IGF-1R plays an imperative role to maintain the normal operation of IGF-1/IGF-1R signaling pathways.

In conclusion, our study highlights the important roles of FUT8 in promoting the proliferation, migration and invasion of trophoblastic cells. The present funding also provides a better understanding of a glycosylation dependent mechanism, which α1,6-fucosylation of IGF-1R is required for the IGF-1/IGF-1R signaling activation in trophoblastic cells. We believe that manipulating FUT8/α1,6-fucosylation may be an effective approach to regulate embryo implantation and placenta development.

Conflicts of interest

The authors declare no competing financial interest.

Author contributions

Ming Yu, Shuai Liu and Qiu Yan designed the experiments. Ming Yu wrote the manuscript. Ming Yu performed Western blot, Lectin blot and Lectin fluorescent staining analysis. Xinyuan Cui and Hao Wang performed cell culture. Jianwei Liu performed statistical analysis. Huamin Qin collected the human placental villi tissues. All authors reviewed the manuscript.

Acknowledgements

This work was supported by the China National Natural Science Foundation Grant (No: 31670810) and China Postdoctoral Science Foundation Grant (No: S2018M630292). We are grateful to the Second Affiliated Hospital of Dalian Medical University for tissues collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2018.11.005.

References

[1] O. Guzeloglu-Kayisli, M. Basar, A. Arici, Basic aspects of implantation, Reprod. Biomed. Online 15 (2007) 728–739.
[2] A.M. Sharkey, N.S. Macklon, The science of implantation emerges blinking into the light, Reprod. Biomed. Online 27 (2013) 453–460, https://doi.org/10.1016/j.rbmo.2013.08.005.
[3] C. Ferretti, L. Bruni, V. Dangles-Marie, A.P. Pecking, D. Bellet, Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts, Hum. Reprod. Update 13 (2007) 121–141, https://doi.org/10.1093/humupd/dml048.
[4] G. Unek, A. Ozmen, B.S. Isenlik, E.T. Korgun, The proliferation mechanism of normal and pathological human placentas, Histol. Histopathol. 32 (2017) 339–349, https://doi.org/10.14670/HH-11-832.
[5] L. Tang, G. He, X. Liu, W. Xu, Progress in the understanding of the etiology and predictability of fetal growth restriction, Reproduction 153 (2017) R227–R240, https://doi.org/10.1530/REP-16-0287.
[6] P.K. Lala, P. Nandi, Mechanisms of trophoblast migration, endometrial angiogenesis in preeclampsia: the role of decorin, Cell Adhes. Migrat. 10 (2016) 111–125, https://doi.org/10.1080/19336918.2015.1106669.
[7] M.I. Kokkinos, P. Murthi, R. Wafai, E.W. Thompson, D.F. Newgreen, Cadherins in the human placenta–epithelial-mesenchymal transition (EMT) and placental development, Placenta 31 (2010) 747–755, https://doi.org/10.1016/j.placenta.2010.06.017.
[8] S. Espino Y Sosa, A. Flores-Pliego, A. Espejel-Nunez, D. Medina-Bastidas, F. Vadillo-Ortega, V. Zaga-Clavellina, G. Estrada-Gutierrez, New insights into the role of matrix metalloproteinases in preeclampsia, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18071448.
[9] G. Moser, B. Huppertz, Implantation and extravillous trophoblast invasion: from rare archival specimens to modern biobanking, Placenta 56 (2017) 19–26, https://doi.org/10.1016/j.placenta.2017.02.007.
[10] D. Goldman-Wohl, S. Yagel, Regulation of trophoblast invasion: from normal implantation to pre-eclampsia, Mol. Cell. Endocrinol. 187 (2002) 233–238.
[11] A.P. Moran, A. Gupta, L. Joshi, Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract, Gut 60 (2011) 1412–1425, https://doi.org/10.1136/gut.2010.212704.
[12] J. Costa, Glycoconjugates from extracellular vesicles: structures, functions and emerging potential as cancer biomarkers, Biochim. Biophys. Acta 1868 (2017) 157–166, https://doi.org/10.1016/j.bbcan.2017.03.007.
[13] C.J.P. Jones, J.D. Aplin, Glycosylation at the fetomaternal interface: does the glycocode play a critical role in implantation? Glycoconj. J. 26 (2009) 359–366, https://doi.org/10.1007/s10719-008-9152-6.
[14] D. Dutta, C. Mandal, C. Mandal, Unusual glycosylation of proteins: beyond the

universal sequon and other amino acids, Biochim. Biophys. Acta 1861 (2017) 3096–3108, https://doi.org/10.1016/j.bbagen.2017.08.025.
[15] M. Schneider, E. Al-Shareffi, R.S. Haltiwanger, Biological functions of fucose in mammals, Glycobiology 27 (2017) 601–618, https://doi.org/10.1093/glycob/cwx034.
[16] A. Kondo, W. Li, T. Nakagawa, M. Nakano, N. Koyama, X. Wang, J. Gu, E. Miyoshi, N. Taniguchi, From glycomics to functional glycomics of sugar chains: identification of target proteins with functional changes using gene targeting mice and knock down cells of FUT8 as examples, Biochim. Biophys. Acta 1764 (2006) 1881–1889, https://doi.org/10.1016/j.bbapap.2006.10.011.
[17] X. Wang, J. Gu, E. Miyoshi, K. Honke, N. Taniguchi, Phenotype changes of Fut8 knockout mouse: core fucosylation is crucial for the function of growth factor receptor(s), Methods Enzymol. 417 (2006) 11–22, https://doi.org/10.1016/S0076-6879(06)17002-0.
[18] T. Fukuda, H. Hashimoto, N. Okayasu, A. Kameyama, H. Onogi, O. Nakagawasai, T. Nakazawa, T. Kurosawa, Y. Hao, T. Isaji, T. Tadano, H. Narimatsu, N. Taniguchi, J. Gu, Alpha1,6-fucosyltransferase-deficient mice exhibit multiple behavioral abnormalities associated with a schizophrenia-like phenotype: importance of the balance between the dopamine and serotonin systems, J. Biol. Chem. 286 (2011) 18434–18443, https://doi.org/10.1074/jbc.M110.172536.
[19] X. Wang, S. Inoue, J. Gu, E. Miyoshi, K. Noda, W. Li, Y. Mizuno-Horikawa, M. Nakano, M. Asahi, M. Takahashi, N. Uozumi, S. Ihara, S.H. Lee, Y. Ikeda, Y. Yamaguchi, Y. Aze, Y. Tomiyama, J. Fujii, K. Suzuki, A. Kondo, S.D. Shapiro, C. Lopez-Otin, T. Kuwaki, M. Okabe, K. Honke, N. Taniguchi, Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice, Proc. Natl. Acad. Sci. U. S. A 102 (2005) 15791–15796, https://doi.org/10.1073/pnas.0507375102.
[20] S. Liu, J. Wang, H.-M. Qin, X.-M. Yan, X.-S. Yang, C. Liu, Q. Yan, LIF upregulates poFUT1 expression and promotes trophoblast cell migration and invasion at the fetal-maternal interface, Cell Death Dis. 5 (2014), https://doi.org/10.1038/cddis.2014.335 e1396.
[21] C. Liu, X. Liang, J. Wang, Q. Zheng, Y. Zhao, M.N. Khan, S. Liu, Q. Yan, Protein O-fucosyltransferase 1 promotes trophoblast cell proliferation through activation of MAPK and PI3K/Akt signaling pathways, Biomed. Pharmacother. 88 (2017) 95–101, https://doi.org/10.1016/j.biopha.2017.01.026.
[22] R. Fritz, C. Jain, D.R. Armant, Cell signaling in trophoblast-uterine communication, Int. J. Dev. Biol. 58 (2014) 261–271, https://doi.org/10.1387/ijdb.140011da.
[23] A.D. Lighten, G.E. Moore, R.M. Winston, K. Hardy, Routine addition of human insulin-like growth factor-I ligand could benefit clinical in-vitro fertilization culture, Hum. Reprod. 13 (1998) 3144–3150.
[24] M. Slater, C.R. Murphy, Differential expression of insulin-like growth factors in the uterine epithelium and extracellular matrix during early pregnancy, Matrix Biol. 18 (1999) 579–584.
[25] M. Kabir-Salmani, S. Shiokawa, Y. Akimoto, K. Sakai, S. Nagamatsu, K. Sakai, Y. Nakamura, A. Lotfi, H. Kawakami, M. Iwashita, Alphavbeta3 integrin signaling pathway is involved in insulin-like growth factor I-stimulated human extravillous trophoblast cell migration, Endocrinology 144 (2003) 1620–1630, https://doi.org/10.1210/en.2002-220886.
[26] W. Jeong, G. Song, F.W. Bazer, J. Kim, Insulin-like growth factor I induces proliferation and migration of porcine trophectoderm cells through multiple cell signaling pathways, including protooncogenic protein kinase 1 and mitogen-activated protein kinase, Mol. Cell. Endocrinol. 384 (2014) 175–184, https://doi.org/10.1016/j.mce.2014.01.023.
[27] M. Kabir-Salmani, S. Shiokawa, Y. Akimoto, H. Hasan-Nejad, K. Sakai, S. Nagamatsu, K. Sakai, Y. Nakamura, A. Hosseini, M. Iwashita, Characterization of morphological and cytoskeletal changes in trophoblast cells induced by insulin-like growth factor-I, J. Clin. Endocrinol. Metab. 87 (2002) 5751–5759, https://doi.org/10.1210/jc.2002-020550.
[28] M. Dews, M. Prisco, F. Peruzzi, G. Romano, A. Morrione, R. Baserga, Domains of the insulin-like growth factor I receptor required for the activation of extracellular signal-regulated kinases, Endocrinology 141 (2000) 1289–1300, https://doi.org/10.1210/endo.141.4.7414.
[29] A.M. Vincent, E.L. Feldman, Control of cell survival by IGF signaling pathways, Growth Hormone IGF Res. 12 (2002) 193–197.
[30] K. Forbes, V.K. Shah, K. Siddals, J.M. Gibson, J.D. Aplin, M. Westwood, Statins inhibit insulin-like growth factor action in first trimester placenta by altering insulin-like growth factor 1 receptor glycosylation, Mol. Hum. Reprod. 21 (2015) 105–114, https://doi.org/10.1093/molehr/gau093.
[31] N.J. Hannan, P. Paiva, E. Dimitriadis, L.A. Salamonsen, Models for study of human embryo implantation: choice of cell lines? Biol. Reprod. 82 (2010) 235–245, https://doi.org/10.1095/biolreprod.109.077800.
[32] S.K. Gupta, P. Bansal, A. Ganguly, B. Bhandari, K. Chakrabarti, Human zona pellucida glycoproteins: functional relevance during fertilization, J. Reprod. Immunol. 83 (2009) 50–55, https://doi.org/10.1016/j.jri.2009.07.008.
[33] F. Poirier, S. Kimber, Cell surface carbohydrates and lectins in early development, Mol. Hum. Reprod. 3 (1997) 907–918.
[34] J.D. Aplin, P.T. Ruane, Embryo-epithelium interactions during implantation at a glance, J. Cell Sci. 130 (2017) 15–22, https://doi.org/10.1242/jcs.175943.
[35] N. Taniguchi, Y. Kizuka, Glycans and cancer: role of N-glycans in cancer biomarker, progression and metastasis, and therapeutics, Adv. Cancer Res. 126 (2015) 11–51, https://doi.org/10.1016/bs.acr.2014.11.001.
[36] W.-C. Liao, C.-H. Liu, C.-H. Chen, W.-M. Hsu, Y.-Y. Liao, H.-M. Chang, C.-T. Lan, M.-C. Huang, M.-K. Shyu, beta-1,4-Galactosyltransferase III suppresses extravillous trophoblast invasion through modifying beta1-integrin glycosylation, Placenta 36 (2015) 357–364, https://doi.org/10.1016/j.placenta.2015.01.008.
[37] W.-C. Liao, C.-H. Chen, C.-H. Liu, M.-J. Huang, C.-W. Chen, J.-S. Hung, C.-H. Chou, C.-H. Chen, M.-I. Che, H.-M. Chang, C.-T. Lan, H.-C. Huang, G.-F. Tseng, M.-K. Shyu, M.-C. Huang, Expression of GALNT2 in human extravillous trophoblasts and its suppressive role in trophoblast invasion, Placenta 33 (2012) 1005–1011, https://doi.org/10.1016/j.placenta.2012.08.007.
[38] E. Yamamoto, K. Ino, E. Miyoshi, K. Inamori, A. Abe, S. Sumigama, A. Iwase, H. Kajiyama, K. Shibata, A. Nawa, F. Kikkawa, N-acetylglucosaminyltransferase V regulates extravillous trophoblast invasion through glycosylation of alpha5beta1 integrin, Endocrinology 150 (2009) 990–999, https://doi.org/10.1210/en.2008-1005.
[39] M. Zhang, M. Wang, R. Gao, X. Liu, X. Chen, Y. Geng, Y. Ding, Y. Wang, J. He, Altered beta1,6-GlcNAc and bisecting GlcNAc-branched N-glycan on integrin beta1 are associated with early spontaneous miscarriage in humans, Hum. Reprod. 30 (2015) 2064–2075, https://doi.org/10.1093/humrep/dev153.
[40] S. Liu, X. Yang, Y. Liu, X. Wang, Q. Yan, sLeX/L-selectin mediates adhesion in vitro implantation model, Mol. Cell. Biochem. 350 (2011) 185–192, https://doi.org/10.1007/s11010-010-0697-x.
[41] J. Roth, C. Zuber, S. Park, I. Jang, Y. Lee, K.G. Kysela, V. Le Fourn, R. Santimaria, B. Guhl, J.W. Cho, Protein N-glycosylation, protein folding, and protein quality control, Mol. Cell. 30 (2010) 497–506, https://doi.org/10.1007/s10059-010-0159-z.
[42] J. Gu, T. Isaji, Q. Xu, Y. Kariya, W. Gu, T. Fukuda, Y. Du, Potential roles of N-glycosylation in cell adhesion, Glycoconj. J. 29 (2012) 599–607, https://doi.org/10.1007/s10719-012-9386-1.
[43] M. Takahashi, Y. Kuroki, K. Ohtsubo, N. Taniguchi, Core fucose and bisecting GlcNAc, the direct modifiers of the N-glycan core: their functions and target proteins, Carbohydr. Res. 344 (2009) 1387–1390, https://doi.org/10.1016/j.carres.2009.04.031.
[44] X. Wang, J. Gu, H. Ihara, E. Miyoshi, K. Honke, N. Taniguchi, Core fucosylation regulates epidermal growth factor receptor-mediated intracellular signaling, J. Biol. Chem. 281 (2006) 2572–2577, https://doi.org/10.1074/jbc.M510893200.
[45] P. Agrawal, B. Fontanals-Cirera, E. Sokolova, S. Jacob, C.A. Vaiana, D. Argibay, V. Davalos, M. McDermott, S. Nayak, F. Darvishian, M. Castillo, B. Ueberheide, I. Osman, D. Fenyo, L.K. Mahal, E. Hernando, A systems biology approach identifies FUT8 as a driver of melanoma metastasis, Cancer Cell 31 (2017) 804–819, https://doi.org/10.1016/j.ccell.2017.05.007 e7.
[46] C.-F. Tu, M.-Y. Wu, Y.-C. Lin, R. Kannagi, R.-B. Yang, FUT8 promotes breast cancer cell invasiveness by remodeling TGF-beta receptor core fucosylation, Breast Cancer Res. 19 (2017) 111, https://doi.org/10.1186/s13058-017-0904-8.
[47] V. Vanhooren, S. Dewaele, M. Kuro-O, N. Taniguchi, L. Dolle, L.A. van Grunsven, E. Makrantonaki, C.C. Zouboulis, C.C. Chen, C. Libert, Alteration in N-glycomics during mouse aging: a role for FUT8, Aging Cell 10 (2011) 1056–1066, https://doi.org/10.1111/j.1474-9726.2011.00749.x.
[48] C. Ganeff, G. Chatel, C. Munaut, F. Frankenne, J.-M. Foidart, R. Winkler, The IGF system in in-vitro human decidualization, Mol. Hum. Reprod. 15 (2009) 27–38, https://doi.org/10.1093/molehr/gan073.
[49] G.D. Agrogiannis, S. Sifakis, E.S. Patsouris, A.E. Konstantinidou, Insulin-like growth factors in embryonic and fetal growth and skeletal development (Review), Mol. Med. Rep. 10 (2014) 579–584, https://doi.org/10.3892/mmr.2014.2258.
[50] Y.-J. Kang, M. Lees, L.C. Matthews, S.J. Kimber, K. Forbes, J.D. Aplin, MiR-145 suppresses embryo-epithelial juxtacrine communication at implantation by modulating maternal IGF1R, J. Cell Sci. 128 (2015) 804–814, https://doi.org/10.1242/jcs.164004.
[51] J. Baker, M.P. Hardy, J. Zhou, C. Bondy, F. Lupu, A.R. Bellve, A. Efstratiadis, Effects of an Igf1 gene null mutation on mouse reproduction, Mol. Endocrinol. 10 (1996) 903–918, https://doi.org/10.1210/mend.10.7.8813730.
[52] M.J.E. Walenkamp, H.J. van der Kamp, A.M. Pereira, S.G. Kant, H.A. van Duyvenvoorde, M.F. Kruithof, M.H. Breuning, J.A. Romijn, M. Karperien, J.M. Wit, A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor, J. Clin. Endocrinol. Metab. 91 (2006) 3062–3070, https://doi.org/10.1210/jc.2005-1597.
[53] T. Kruis, J. Klammt, A. Galli-Tsinopoulou, T. Wallborn, M. Schlicke, E. Muller, J. Kratzsch, A. Korner, R. Odeh, W. Kiess, R. Pfaffle, Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation, J. Clin. Endocrinol. Metab. 95 (2010) 1137–1142, https://doi.org/10.1210/jc.2009-1433.
[54] C.J. Green, S.T. Fraser, M.L. Day, Insulin-like growth factor 1 increases apical fibronectin in blastocysts to increase blastocyst attachment to endometrial epithelial cells in vitro, Hum. Reprod. 30 (2015) 284–298, https://doi.org/10.1093/humrep/deu309.
[55] C. Zhu, X. Qi, Y. Chen, B. Sun, Y. Dai, Y. Gu, PI3K/Akt and MAPK/ERK1/2 signaling pathways are involved in IGF-1-induced VEGF-C upregulation in breast cancer, J. Canc. Res. Clin. Oncol. 137 (2011) 1587–1594, https://doi.org/10.1007/s00432-011-1049-2.
[56] C. Worrall, D. Nedelcu, J. Serly, N. Suleymanova, I. Oprea, A. Girnita, L. Girnita, Novel mechanisms of regulation of IGF-1R action: functional and therapeutic implications, Pediatr. Endocrinol. Rev. 10 (2013) 473–484.