Dysregulated GLUT1 May Be Involved in the Pathogenesis of Preeclampsia by Impairing Decidualization

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Research

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Abstract

Background: Preeclampsia (PE), a hypertensive complication in pregnancy, is a major contributor to maternal and fetal morbidity and mortality. Thus far, the molecular mechanism underlying PE has not been investigated thoroughly. Glucose transporter 1 (GLUT1) is a central rate-limiting pump for glucose uptake and subsequent utilization. Our previous RNA-seq results demonstrated it was significantly downregulated in deciduas from severe PE patients. Therefore, we aimed to explore the role of GLUT1 in the occurrence of PE.

Methods: In this study, GLUT1 levels were evaluated by quantitative PCR, Western blotting and immunohistochemical staining in severe preeclamptic deciduas. The levels of GLUT1 during decidualization were also studied in human endometrial stromal cells (hESCs). Moreover, the role of GLUT1 during decidualization was studied by *GLUT1*-siRNA treatment. Furthermore, we explored the regulatory role of miRNA in GLUT1 expression.

Results: The expression of GLUT1 was significantly downregulated in the deciduas from severe PE patients. Additionally, the level of GLUT1 was substantially induced in hESCs during *in vitro* decidualization. Moreover, *GLUT1* knockdown significantly reduced the mRNA levels of decidualization markers (*IGFBP1* and *PRL*) and aerobic glycolysis-related genes (*LDHA* and *MCT4*), and decreased glucose uptake and lactate production. Furthermore, the levels of apoptotic genes *P53*, *P21* and *BAX* increased whereas the levels of *BCL2* decreased after *GLUT1* knockdown. Target prediction results and luciferase analysis showed *GLUT1* is one of the targets of miR-140-5p, which is partly responsible for the impaired GLUT1 level.

Conclusion: These results demonstrate that GLUT1 exerts pivotal role in human decidualization by participating in glycolysis, and its deficiency may trigger aberrant glycolysis and thus leads to destructive decidualization, which may be a pathogenetic mechanism of PE. These data suggest GLUT1 might be a promising target for PE therapy.

Introduction

Preeclampsia (PE) is an obstetric disorder affecting 5–10% of all pregnancies worldwide, especially in developing countries, and it is a major contributor to considerable maternal and perinatal morbidity and mortality [1]. PE is generally characterized by new onset hypertension, and/or proteinuria after 20 weeks of gestation, complicated by maternal kidney injury, stroke and even death [2], and/or poor fetal consequences (for example, preterm delivery, intrauterine growth restriction and stillbirth) [3]. Severe PE (sPE) was diagnosed as systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 110 mm Hg, accompanied by systemic disturbances such as thrombocytopenia, liver dysfunction, renal insufficiency, and cerebral or visual disturbances [2]. It is well established that the placenta is an important determinant in the development of PE since the delivery of the placenta is the exclusively effective management of PE

[4]. Despite myriads of investigations, the precise etiologic factors of PE remain elusive because of its heterogeneity [5].

At mid-secretory phase of a menstrual cycle, the elongated spindle-shaped human endometrial stromal cells transform into epithelioid decidual cells, a process termed decidualization [6, 7]. During decidualization, the morphological reprogramming of the endometrial stromal compartment is accompanied by integrated changes at both the transcriptome and proteome levels. Prolactin (PRL) and insulin-like growth factor binding protein 1 (IGFBP1), which are abundantly triggered in this process, have been widely recognized as decidualization biomarkers [8]. It is generally acknowledged that progesterone (P_4) initiates and drives the progression of decidualization of estrogen-primed endometrium through activating nuclear progesterone receptor (PR) [9] Additionally, the sophisticated and coordinated interplay between P_4 / PR and cAMP/PKA pathway exists in the decidualization process [9]. The precisely synchronous development of the “seed”, an adequate embryo, and the “soil”, decidua is a prerequisite for embryo implantation, placentation and pregnancy maintenance[10]. Defective decidualization would cause adverse obstetric outcomes including PE. Our recent studies highlight the causative role of the abnormal decidualization in the progression of PE [11–13], and this view has been confirmed by other researches [14, 15]. Nevertheless, our understanding of the association between abnormal decidualization and PE is just emerging.

Glucose transporter 1 (GLUT1), the first rate-limiting enzyme in glucose metabolism, is responsible for basal glucose uptake in most cell types [16]. There is evidence that GLUT1-dependent glycolysis is a typical hallmark of various cancers, even in the presence of oxygen [17]. This phenomenon was first observed by Otto Warburg in 1924 and is therefore termed as Warburg effect, also known as aerobic glycolysis [17]. Earlier reports suggested that Warburg-like glycolysis is implicated in the differentiation of endometrial stroma into decidua, demonstrated by the high accumulation of glycogen and activated glycolysis-related factors including GLUT1 [18]. Dysregulated GLUT1 in decidua has been described in several obstetric complications such as idiopathic infertility [19]. Our previous transcriptome results also show significantly decreased GLUT1 in the sPE decidua [20]. However, the molecular mechanism by which GLUT1 was involved in decidualization was not clearly understood.

Mammalian cells use glucose as their main power for proliferation [21]. As a glucose uptake pump, GLUT1 downregulation causes reduced glucose availability, which has been reported to be associated with cell cycle and apoptosis events through the activation of proapoptotic molecules P53 and BAX [22–25]. Therefore, we supposed that GLUT1 downregulation in decidual cells might jeopardize decidualization through the activation of cell apoptosis in PE.

MicroRNAs (miRNAs) are small RNA containing 20–24 nucleotides. Seven nucleotides in the second to eighth position of miRNA (the seed region) complementarily bind to the nucleotides in target mRNA estimated to downregulate 30% of protein-coding genes [26]. MiRNAs play essential roles in cell proliferation, cell cycle and cell apoptosis [27]. Dysregulated miRNAs are involved in multiple diseases. Our recent findings showed several miRNAs are implicated in regulating the metabolism of PE [28, 29].

Therefore, we suppose that GLUT1 might also be regulated by some miRNAs, and the results demonstrated the decreased *GLUT1* resulting from upregulated miR-140-5p activates apoptotic pathway and then contributes to the defective decidualization, which may work as partial aetiology of PE.

Materials And Methods

Study population and tissue collection

Twenty normal pregnant (NP) women and 20 severe preeclampsia (sPE) patients were recruited from Ji'nan Maternity and Child Care Hospital between June 2016 and July 2017 in this study. All subjects were single pregnancies, and women suffered from chronic hypertension, diabetes mellitus, renal disease and other pregnancy related disorders were excluded. sPE was diagnosed according to the American College of Obstetricians and Gynecologists [2]. By definition, the systolic pressure blood was ≥ 160 mm Hg or diastolic blood pressure ≥ 110 mm Hg on two occasions ≥ 4 h apart. Proteinuria was present as ≥ 3 grams in 24 h urine collection (≥ 2 + by dipstick). Sampling was carried out by scrubbing the uterus from the site of implantation as soon as the placenta was delivered by caesarean section using sterile gauze. The decidual tissues were washed with sterile saline to remove blood, snap frozen in liquid nitrogen and stored at -80°C for further processing. The clinical characteristics of the participants are summarized in Table S1.

Immunohistochemistry

Decidual tissues were embedded in O.C.T. compound (Sakura Tissue-Tek Xpress, Torrance, CA, USA) and cut into 5-μm thick cryosections. The labeled slides were fixed in ice-cold acetone for 10 min at -20°C, and rinsed in phosphate-buffered saline (PBS) at room temperature every five min with three changes of buffer [30]. Thereafter, the sections were immersed in absolute methanol containing 3% H_2O_2 to eliminate endogenous peroxidase activity. The tissues were probed with polyclonal primary antibody against GLUT1 (sc-7903; Santa Cruz Biotechnology, CA, USA) at 1:100 dilution overnight at 4°C. Pre-immune IgG rather than primary antibody was used for negative control. After washing with PBS, the specimens were incubated with horseradish peroxidase-conjugated secondary antibodies diluted 1:200 at 37°C for 1 h. Following three additional 5 min washing in PBS, the sections were stained with diaminobenzidine (DAB) (ZLI-9033, ZSGB-BIO, Beijing, China), counterstained with hematoxylin, dehydrated through ethanols, and hyalinized in xylene, then the sections were mounted with neutral gum and examined using a Digital Scanning System (Pannoramic MIDI, 3DHISTECH, Budapest, Hungary) [31]. Immunohistochemical staining was quantified with Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA), and for each group, total of 36 fields from 12 different women (each woman 3 fields) were quantified and analyzed.

Total RNA extraction and quantitative PCR

Total RNA was extracted from decidual tissues and human endometrial stromal cells (hESCs) using TRNzol reagent (Tiangen, Beijing, China) and animal total RNA isolation kit (Foregene, Chengdu, China)

respectively according to manufacturer's instructions. RNA samples (500 ng) were reverse transcribed into cDNA using FastQuant RT Kit with gDNase (Tiangen). As for miRNA, the synthesis of cDNA was performed using Mir-X™ miRNA First-Strand Synthesis Kit (Takara, Dalian, China). Quantitative PCR was carried out using SuperReal PreMix Plus Kit (Tiangen) in Light Cycler 96 system (Roche, Basel, Switzerland). Quantitative PCR was conducted using the following procedures: pre-denaturation for 5 min at 95°C, 40 cycles of 10 s at 95°C, 20 s at 58°C, 20 s at 72°C and a final extension for 5 min at 72°C. The primers used are listed in Table S2. The mRNA and miRNA levels were analyzed using the ΔΔCt method with normalization to reference genes *ACTB* and *U6* respectively [6]. All samples were performed in triplicate in one assay.

Protein extraction and Western blotting

Total protein was extracted using radio immunoprecipitation assay lysis buffer (Beyotime, Shanghai, China) with protease inhibitors (Sigma Chemical Co., St. Louis, MO, USA). Protein extracts (30 μg) were subjected to SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocked with 5% non-fat milk, the membranes were incubated overnight at 4°C with primary antibodies against GLUT1 (1:2000 dilution; sc-7903, Santa Cruz Biotechnology) or β-actin (1:5000 dilution; sc-47778, Santa Cruz Biotechnology). Subsequently, the membranes were probed with horseradish peroxidase-conjugated secondary antibodies at 1:5000 dilutions for 1 h at room temperature. At the end, the chromogenic reaction was performed by incubating the membranes with enhanced chemoluminescence (ECL) detection reagents (Millipore). The images were acquired with a Gel-Imaging Detection System (5500 Multi, Tanon, Shanghai, China). Densitometric analysis was conducted by using QuantiScan software (Biosoft, Cambridge, UK) [32].

Cell culture, decidualization in vitro and transfection

The immortalized hESCs were from the American Type Culture Collection (ATCCR CRL-4003TM). The cells were maintained in phenol red-free DMEM/F12 medium (Gibco, NY, USA) supplemented with 10% charcoal-stripped FBS (BI, Beit Haemek, Israel) at 37°C under 5% CO_2. HESCs were treated with 0.5 mmol/L db-cAMP (Sigma Chemical Co.,) and 1 μmol/L P_4 (Meilun Biotechnology Co., Dalian, China) simultaneously to induce decidualization *in vitro*. *GLUT1* siRNA, miRNA mimics and inhibitor were synthesized by RiboBio (RiboBio Co., Guangzhou, China). When the cells reached 60% confluence, transient transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Subsequently, these cells were harvested for quantitative PCR and Western blotting analysis.

Glucose uptake and lactate production assay

Glucose uptake was measured using glucose oxidase method kit (GOD, Applygen Technologies, China) according to the manufacturers' instructions. Briefly, the cells were grown in starvation medium overnight and were then stimulated with 1 μmol/L insulin for 30 min. 48 h later, the supernatants were harvested

and the optical density was measured at 550 nm using microplate reader (Spectra Max M5, Molecular Devices, USA).

For lactate assay, the cell supernatants were harvest after transfection with *GLUT1*-siRNA. After centrifugation, 20 μL of supernatants were used to analyze the optical density at 530 nm using Lactic Acid assay kit (Jianchengbio, Nanjing, China) according to the manufacturers' instructions.

Dual-luciferase reporter assays

GLUT1 3'-UTR wild-type fragment containing miR-140-5p binding sites was inserted into a pmirGLO dual-luciferase miRNA target expression vector (Promega, WI, USA). The cells were transfected with miR-140-5p mimics along with *GLUT1* wild-type (WT) or mutant constructs (MUT) using lipofectamine 3000 reagent (Invitrogen, CA). Luciferase activity was assayed after 48 h transfection using dual-luciferase reporter assay system (Promega). The renilla luciferase activity was used to normalize the activity of firefly luciferase.

Statistical analysis

Data analysis was performed using SPSS 24.0 software (SPSS, Chicago, IL, USA). All data are expressed as mean ± SEM and all experiments were independently performed at least three times. Log transformation was applied to change the scattered distribution of miRNA data. Comparison between two groups was carried out using Student's *t*-test and differences with $P < 0.05$ were considered as statistically significant. In addition, Pearson correlation analysis was carried out to evaluate the relationship between the levels of miRNA and *GLUT1*.

Results

The clinical data of the study population

The clinical characteristics of all participants are shown in Table S1. No significant difference in maternal age was observed between the cases and controls ($P = 0.075$). The gestational age and the birth weight of the cases were significantly shorter than those of the controls ($P < 0.001$). Conversely, the systolic and diastolic blood pressures were significantly higher in the sPE group compared to control group ($P < 0.001$). Additionally, severe proteinuria was detected in sPE group, whereas normal pregnant women had no signs of proteinuria.

The expression of GLUT1 in decidual tissues from cases and controls

To explore GLUT1 expression in decidual tissues, we performed quantitative PCR and Western blotting. As the data demonstrated, the mRNA level of *GLUT1* remained relatively lower in cases compared with controls (Fig. 1A), which were in accord with the GLUT1 protein level (Fig. 1B, C). Moreover, immunohistochemical staining revealed a notable decrease of GLUT1 expression in cases compared with controls (Fig. 1D, E).

The involvement of GLUT1 in decidualization

To explore the expression of GLUT1 during decidualization, hESCs were subject to decidualization in the presence of P_4 and db-cAMP. The morphological differences in hESCs during decidualization were evaluated. As depicted in Fig. S1A and B, the untreated hESCs were elongated and long fusiform. With the progression of induction, the cells became larger and polygon-shaped (Fig. S1C, D). On day 6 of induction, the cells of both groups were more confluent, and the treated cells developed relatively rounded appearance (Fig. S1E, F). Apart from the morphology change, the mRNA levels of decidualization markers *IGFBP1* and *PRL* were significantly increased on day 3 and day 6 of treatment (Fig. 2A, B). Meanwhile, the levels of *GLUT1* increased prominently on day 3 and were further upregulated on day 6 (Fig. 2C). Moreover, GLUT1 protein level also increased as revealed by Western blotting (Fig. 2D, E). Furthermore, lactate dehydrogenase A (LDHA) and monocarboxylate transporter 4 (MCT4), which are responsible for converting pyruvate into lactate and exporting lactate respectively [33], were also increased during decidualization (Fig. 2F).

GLUT1 knockdown impaired glycolysis and the decidualization of hESCs

To explore the role of GLUT1 in glycolysis of hESCs, we knocked *GLUT1* down using siRNA oligos. Efficient *GLUT1* knockdown was verified by quantitative PCR and Western blotting (Fig. 3A-C). Additionally, the glucose uptake and lactate production of hESCs were suppressed by *GLUT1* knockdown (Fig. 3D, E). To further confirm whether GLUT1 was required for the decidualization of hESCs, we induced decidualization after knocking *GLUT1* down. The results showed the mRNA level of decidualization markers *IGFBP1* and *PRL* was downregulated significantly after *GLUT1* siRNA transfection (Fig. 3F). Collectively, these results established an indispensable role of GLUT1 in glycolysis and the decidualization of hESCs.

The activation of apoptotic pathways in hESCs after GLUT1 knockdown

The afore-mentioned findings drove us to characterize the molecular basis underlying the deficient decidualization of hESCs after *GLUT1* knockdown. We firstly used STRING database to predict the proteins interacted with GLUT1. The predicted proteins are involved in canonical cell cycle and apoptotic pathway (Fig. 4A). Next, we selected cell cycle and apoptotic pathway factors P53 (TP53), P21 (CDKN1A), BCL2 and BAX to verify their mRNA levels by quantitative PCR. The results demonstrated the mRNA levels of cell cycle related genes *P53* and *P21* were elevated (Fig. 4B, C). As for apoptotic genes, *BCL2* level decreased, whereas the transcription of *BAX* dramatically increased after *GLUT1* siRNA treatment (Fig. 4D).

The identification of miRNAs regulating GLUT1

To elucidate the molecular mechanism of GLUT1 downregulation, we explored the regulatory role of the miRNA in GLUT1 expression. TargetScan, Pictar, DIANA and miRanda were used to predict the potential miRNAs targeting *GLUT1*. As shown in Fig. S2A-C, bioinformatics analysis revealed the seed sequences of three candidate miRNAs, miR-130b-3p, miR-140-5p and miR-328-3p, which are complementary to the

nucleotide sequences of 3'-UTR of *GLUT1* mRNA. Moreover, quantitative PCR data suggested on day 3 and day 6 of induction, the levels of miR-130b-3p, miR-140-5p and miR-328-3p were significantly reduced (Fig. 4E-G). Furthermore, there were apparent inverse linear relationships between miR-130b-3p and *GLUT1* levels (Pearson correlation coeffcient $r = 0.753$; $P < 0.01$), miR-140-5p and *GLUT1* levels ($r = 0.788$; $P < 0.01$), and miR-328-3p and *GLUT1* levels ($r = 0.735$; $P < 0.01$) (Fig. 4H-J).

MiR-140-5p downregulates GLUT1 by directly targeting its 3'-UTR in hESCs

Next, we explored the expression of the above-mentioned miRNAs in sPE decidua and found that miR-140-5p was markedly increased (Fig. 5A), whereas the expressions of miR-130b-3p and miR-328-3p showed no significant changes (Fig. S2D, E). To further demonstrate the direct interactions between miRNAs and GLUT1, we transfected hESCs with the mimics of the three above mentioned miRNAs respectively and analyzed the mRNA level of *GLUT1*, *IGFBP1* and *PRL*. As shown in Fig. 5B and Fig. S2F, G, only after miR-140-5p mimics transfection, the mRNA levels of *GLUT1*, *IGFBP1* and *PRL* decreased significantly. Besides, we found miR-140-5p had three potential binding sites in the 3'-UTR of *GLUT1* mRNA by sequence alignment (Fig. S2H). Therefore, we picked miR-140-5p out, and further confirmed its role by miR-140-5p inhibitor transfection. The results showed miR-140-5p inhibitor could enhance the mRNA levels of *GLUT1*, *IGFBP1* and *PRL* (Fig. 5C, D). In addition, the results also demonstrated miR-140-5p mimics suppressed the protein level of *GLUT1* whereas miR-140-5p inhibitor enhanced the protein level of *GLUT1* (Fig. 5E-H). To further explore whether *GLUT1* is a direct target of miR-140-5p in hESCs, *GLUT1*-MUT and WT constructs were generated (Fig. 5I). Our results showed miR-140-5p mimics significantly decreased the relative luciferase activity of *GLUT1*-WT by about 50% compared with negative control (Fig. 5J), whereas the relative luciferase activity was not influenced by the *GLUT1*-MUT construct. These data therefore indicated *GLUT1* is one of the direct targets of miR-140-5p in hESCs.

The effects of miR-140-5p on the glycolytic metabolism and decidualization in hESCs

As downstream factors of GLUT1 in glycolytic pathway, LDHA and MCT4 are responsible for converting pyruvate into lactate and exporting lactate respectively [33]. Besides, IGFBP1 and PRL are acknowledged decidualization markers [8]. To explore the effects of miR-140-5p on glycolytic metabolism and decidualization in hESCs, the mRNA levels of *LDHA*, *MCT4, IGFBP1* and *PRL* were examined. As shown in Fig. 6A-D, the mRNA levels of *LDHA* and *MCT4* were decreased after miR-140-5p mimics transfection, whereas miR-140-5p inhibitor enhanced the mRNA levels of *LDHA* and *MCT4*. Besides, *LDHA* and *MCT4* expression was reduced after *GLUT1* siRNA transfection. However, the levels of *LDHA* and *MCT4* could be rescued by co-transfecting hESCs with miR-140-5p inhibitor and *GLUT1* siRNA. Collectively, these results revealed miR-140-5p could impair the GLUT1-dependent glycolytic metabolism and decidualization in hESCs.

Discussion

In this study, we investigated the expression and the potential role of GLUT1 in decidual tissue and its relationship with PE. Our data suggested the mRNA and protein levels of GLUT1 were significantly

decreased in preeclamptic decidua. During *in vitro* decidualization, the expression of decidual-specific genes *IGFBP1* and *PRL* increased accompanied by strong induction of *GLUT1* and glycolytic genes *LDHA* and *MCT4*, Whereas *GLUT1* knockdown led to dramatic reduced *IGFBP1*, *PRL*, glucose uptake and lactate production. Moreover, the levels of canonical apoptotic genes *BCL2* reduced and *BAX* increased significantly. Additionally, the results demonstrated that *GLUT1* was negatively regulated by miR-140-5p. We conclude that GLUT1 which is affected by aberrant miRNA is an important player in decidualization through participating in glycolysis, and its deficiency may attenuate glycolysis and then cause suboptimal decidual response, which is possibly related to PE.

It is well established that PE severely threatens maternal and offspring's health and causes multiple organ dysfunction. It is generally accepted that placenta is a key to the development of PE and PE is a placental origin disorder to a certain extent [4]. Nevertheless, the growth and function of placenta is regulated by decidua. During placentation, blastocyst derived trophoblast cells differentiate into villous trophoblast and extravillous trophoblast cells [34]. The latter cells which are indispensable cellular component of placenta invade deeply into uterine spiral arteries and decidual stroma [34] where the decidual cells regulate the differentiation, the invasion and the survival of extravillous trophoblast cells [34]. In other words, decidua is the basis for placenta genesis. Therefore, we have been focused on the role of decidua in the pathogenesis of PE.

Previous studies have demonstrated that decidualizing hESCs undergo a morphological differentiation resembling mesenchymal-epithelial transition [7], which needs accelerated glycolysis for the changes of cellular morphology, and for the increasing transcriptome and proteome levels to supply the embedded fetus [8, 33]. In addition, the biochemical alterations, including the production of decidual cell-specific proteins, the aggregation of glycogen and lipid droplets in the cytoplasm and the growth of uterine spiral arteries [7] all require activated Warburg-like glycolysis to supply energy or metabolic intermediates [33]. At the same time, Warburg effect-associated factors are induced during these processes. Our previous studies have also proved that there is glycolysis in the decidua of pregnant women and it is substantially damaged in PE [20], and the glycolytic factors in decidua are critical to the occurrence of PE, including the first kinase hexokinase 2 (HK2) which is downregulated in preeclamptic decidua [28]. The reduced HK2 may contribute to the development of PE by impairing glycolysis and subsequently jeopardizing decidualization. In addition, another important factor in glycolysis, phosphoglycerate kinase 1 (PGK1) which is an ATP-generating enzyme, is also reduced in preeclamptic decidua [29]. The reduced PGK1 impedes glycolysis and angiogenesis in decidua and may be involved in the incidence of PE. In this study, we explored the role of GLUT1, a glucose uptake pump of Warburg effect [16]. Warburg effect is accelerated for bioenergy and metabolites by overexpressing GLUT1 in cancerous cells [17], whereas current results demonstrated the expression of GLUT1 was decreased in the decidua of sPE, indicating Warburg-like glycolysis is impaired in PE. The importance of Warburg-like glycolysis to decidualization has also been highlighted in a finding that intraperitoneal injection of glycolysis inhibitor, 3-bromopyruvate into pregnant mice after implantation reduces the size and weights of implantation sites [33]. The authors also reported that *Glut1* and its downstream factors *Ldha* and *Mct4* are strongly induced during *in vitro* decidualization of mouse endometrial stromal cells [33]. Consistently, our study

showed decidualization markers IGFBP1 and PRL, and glycolytic factors LDHA and MCT4 also increased their expression together with the upregulated GLUT1 in decidualized hESCs. The induced GLUT1 during decidualization accelerates glucose uptake by decidual cells, and thus improves Warburg-like glycolysis.

Lactate is a metabolic product generated in the last step of Warburg-like glycolysis. Its production varies according to glucose uptake [33]. Consistently, we showed lactate production decreased as decreased glucose uptake by *GLUT1*-siRNA during decidualization. Lactate catalyzed by Ldha can be transported out of decidual cells by membrane transporter Mct4 [35]. Importantly, lactate communication universally exists between decidual cells expressing Mct4 and surrounding undifferentiated stromal cells [33]. In this regard, the acidic environment created by exported lactate in decidua has been confirmed to promote the proliferation of undifferentiated stromal cells. The low pH microenvironment also assists decidual extracellular matrix disaggregation to facilitate trophoblast invasion, promotes the accumulation of VEGF in endometrium to induce angiogenesis, and modulates decidual immune response to create local immune tolerance [36]. The inhibition of uterine acidification would disrupt decidualization and implantation process [37]. Therefore, the decreased lactate production might also contribute to the pathogenies of PE.

GLUT1 may also be involved in cell apoptosis. In STRING database, P53, P21, BCL2 and BAX were predicted to interact with GLUT1. Normally, the activity of P53, a crucial cell cycle checkpoint protein, is kept at low levels. However, P53 is activated and causes cell cycle arrest in response to diverse cellular stresses including glucose starvation [22]. It is also reported that activated P53 promotes cell survival under glucose deprivation condition [22]. However, this adaptive response is limited. Persistent cell cycle arrest caused by activated P53 leads to cell apoptosis through directedly activating BAX, and repressing BCL2 transcriptionally [25]. P21 can also be directly activated by P53, and thus inhibits cell proliferation [38]. In this extent, P53 may act as a metabolic sensor and coordinate cell apoptosis in dealing with glucose deprivation. Therefore, the decreased GLUT1 in our results might blunt decidual response through the activation of P53-mediated cell apoptotic pathways. This pathological cell apoptosis, however, differs from normal decidual cell apoptosis which is crucial to successful implantation [39].

The role of miRNAs in regulating decidualization and PE has been reported. Previous study demonstrated that miR-6887-3p competitively binds *HK2P1* to decrease *HK2* in preeclamptic decidua [28], while miR-330-5p competitively binds *PGK1P2* to decrease *PGK1* in the decidua of preeclamptic pregnancies [29]. Converging evidence showed miR-140-5p which functions as a tumor suppressor to inhibit the proliferation and invasion of cancer cells is markedly decreased in several cancers [40, 41]. Additionally, a cross-sectional study reported circulating miR-140-5p increases markedly in type 2 diabetes patients [42], indicating the involvement of miR-140-5p in glucose metabolism. In our study, we demonstrated the increased miR-140-5p downregulated GLUT1-dependent glucose metabolism, and thus caused impaired decidualization and consequently contributed to PE.

Conclusions

Taking together, we proposed the following model for GLUT1 in the development of PE (Fig. 6E). GLUT1 plays an important role in endometrium decidualization. Decreased *GLUT1* caused by increased miR-140-5p in decidua attenuated Warburg-like glycolysis, demonstrated by reduced *LDHA* and *MCT4*, as well as decreased glucose uptake and lactate production. Under such circumstances, P53-associated apoptotic pathway was activated and decidualization was blunted. Our findings also suggest GLUT1 might be a potential target in treating PE.

Abbreviations

sPE, severe preeclampsia; NP, normal pregnant women; GLUT1, glucose transporter 1; IGFBP1, insulin-like growth factor binding protein 1; PRL, prolactin; hESCs, human endometrial stromal cells; P_4, progesterone; db-cAMP, N6, 2´-O-dibutyryladenosine cAMP sodium salt.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Ji'nan Maternity and Child Care Hospital. Written informed consent was obtained from all pregnant women according to the guidelines of the Ministry of Public Health of China.

Consent for publication

Not applicable.

Availability of data and materials

All data generated through this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors′ contributions

CZ and MY designed the study. MY performed the experiments and drafted the manuscript. HL collected the samples. MMR acquired data. HYZ and LLH analyzed and interpreted data. CZ and MY revised the manuscript. All authors read and approved the final manuscript.

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References

1. Turanov AA, Lo A, Hassler MR, Makris A, Ashar-Patel A, Alterman JF, Coles AH, Haraszti RA, Roux L, Godinho B, Echeverria D, Pears S, Iliopoulos J, Shanmugalingam R, Ogle R, Zsengeller ZK, Hennessy A, Karumanchi SA, Moore MJ, Khvorova A. RNAi modulation of placental sFLT1 for the treatment of preeclampsia. Nat Biotechnol. 2018;1164-73.
2. Obstetricians ACo, Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstet gynecol. 2013;122:1122.
3. Wang AM, Karumanchi SA. Offspring Cardiovascular Disease in Preeclampsia: Nature Versus Nurture? Hypertension. 2017;69:589-90.
4. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension. 2008;51:970-5.
5. Roberts JM, Catov JM. Preeclampsia more than 1 disease - Or is it? Hypertension. 2008;51:989-90.
6. Ding N-Z, Qi Q-R, Gu X-W, Zuo R-J, Liu J, Yang Z-M. De novo synthesis of sphingolipids is essential for decidualization in mice. Theriogenology. 2018;106:227-36.
7. Dunn CL, Kelly RW, Critchley HO. Decidualization of the human endometrial stromal cell: an enigmatic transformation. Reprod Biomed Online. 2003;7:151-61.
8. Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin Reprod Med. 2007;25:445-53.
9. Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. Endocr J. 2008;55:795-810.
10. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. Nat Med. 2012;18:1754-67.
11. Wang G, Zhang Z, Chen C, Zhang Y, Zhang C. Dysfunction of WNT4/WNT5A in deciduas: possible relevance to the pathogenesis of preeclampsia. J Hypertens. 2016;34:719-27.
12. Zhang D, Chang X, Bai J, Chen Z-J, Li W-P, Zhang C. The study of cyclooxygenase 2 in human decidua of preeclampsia. Biol reprod. 2016;95:56, 51-8.
13. Zhang Y, Yang J, Lv S, Zhao DQ, Chen ZJ, Li WP, Zhang C. Downregulation of decidual SP1 and P300 is associated with severe preeclampsia. J Mol Endocrinol. 2018;60:133-43.

14. Garrido-Gomez T, Dominguez F, Quinonero A, Diaz-Gimeno P, Kapidzic M, Gormley M, Ona K, Padilla-Iserte P, McMaster M, Genbacev O, Perales A, Fisher SJ, Simon C. Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. P Natl Acad Sci USA. 2017;114:E8468-77.
15. Conrad KP, Rabaglino MB, Uiterweer EDP. Emerging role for dysregulated decidualization in the genesis of preeclampsia. Placenta. 2017;60:119-29.
16. Hruz PW, Mueckler MM. Structural analysis of the GLUT1 facilitative glucose transporter. Mol Membr Biol. 2001;18:183-93.
17. Heiden MGV, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. Science. 2009;324:1029-33.
18. Kommagani R, Szwarc MM, Kovanci E, Gibbons WE, Putluri N, Maity S, Creighton CJ, Sreekumar A, DeMayo FJ, Lydon JP, O'Malley BW. Acceleration of the Glycolytic Flux by Steroid Receptor Coactivator-2 Is Essential for Endometrial Decidualization. Plos Genet. 2013;9:e1005515.
19. Frolova AI, Moley KH. Glucose transporters in the uterus: an analysis of tissue distribution and proposed physiological roles. Reproduction. 2011;142:211-20.
20. Tong J, Zhao WX, Lv H, Li WP, Chen ZJ, Zhang C. Transcriptomic Profiling in Human Decidua of Severe Preeclampsia Detected by RNA Sequencing. J Cell Biochem. 2018;119:607-15.
21. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Gene Dev. 2004;18:1272-82.
22. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ, Thompson CB. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell. 2005;18:283-93.
23. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol. 2003;23:7315-28.
24. Li SN, Li JJ, Dai WQ, Zhang QH, Feng J, Wu LW, Liu T, Yu Q, Xu SZ, Wang WW, Lu XY, Chen K, Xia YJ, Lu J, Zhou YQ, Fan XM, Mo WH, Xu L, Guo CY. Genistein suppresses aerobic glycolysis and induces hepatocellular carcinoma cell death. Brit J Cancer. 2017;117:1518-28.
25. Okoshi R, Ozaki T, Yamamoto H, Ando K, Koida N, Ono S, Koda T, Kamijo T, Nakagawara A, Kizaki H. Activation of AMP-activated protein kinase induces p53-dependent apoptotic cell death in response to energetic stress. J Biol Chem. 2008;283:3979-87.
26. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120:15-20.
27. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136:215-33.
28. Lv H, Tong J, Yang JQ, Lv SJ, Li WP, Zhang C, Chen ZJ. Dysregulated Pseudogene HK2P1 May Contribute to Preeclampsia as a Competing Endogenous RNA for Hexokinase 2 by Impairing Decidualization. Hypertension. 2018;71:648-58.

29. Tong J, Yang JQ, Lv H, Lv SJ, Zhang C, Chen ZJ. Dysfunction of pseudogene PGK1P2 is involved in preeclampsia by acting as a competing endogenous RNA of PGK1. Pregnancy Hypertens. 2018;13:37-45.
30. Cui LL, Yang G, Pan J, Zhang C. Tumor necrosis factor alpha knockout increases fertility of mice. Theriogenology. 2011;75:867-76.
31. Wang N, Li H, Zhu Y, Li N, Chen Z-J, Zhang C. Melatonin protects against Epirubicin-induced ovarian damage. J Reprod Develop. 2019;2017-88.
32. Meng X-q, Dai Y-y, Jing L-d, Bai J, Liu S-z, Zheng K-g, Pan J. Subcellular localization of Pyk2 during oocyte fertilization and early-embryo development in mice. J Reprod Develop 2016;62:351-8.
33. Zuo RJ, Gu XW, Qi QR, Wang TS, Zhao XY, Liu JL, Yang ZM. Warburg-like Glycolysis and Lactate Shuttle in Mouse Decidua during Early Pregnancy. J Biol Chem. 2015;290:21280-91.
34. Velicky P, Knofler M, Pollheimer J. Function and control of human invasive trophoblast subtypes: Intrinsic vs. maternal control. Cell Adhes Migr. 2016;10:154-62.
35. Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest. 2013;123:3685-92.
36. Ma LN, Huang XB, Muyayalo KP, Mor G, Liao AH. Lactic Acid: A Novel Signaling Molecule in Early Pregnancy? Front Immunol. 2020;11:279.
37. Xiao S, Li R, El Zowalaty AE, Diao HL, Zhao F, Choi YW, Ye XQ. Acidification of uterine epithelium during embryo implantation in mice. Biol Reprod. 2017;96:232-43.
38. Neganova I, Zhang X, Atkinson S, Lako M. Expression and functional analysis of G1 to S regulatory components reveals an important role for CDK2 in cell cycle regulation in human embryonic stem cells. Oncogene. 2009;28:20-30.
39. Boeddeker SJ, Hess AP. The role of apoptosis in human embryo implantation. J Reprod Immunol. 2015;108:114-22.
40. Hu Y, Li YY, Wu C, Zhou L, Han XX, Wang QY, Xie XS, Zhou YX, Du ZW. MicroRNA-140-5p inhibits cell proliferation and invasion by regulating VEGFA/MMP2 signaling in glioma. Tumor Biol. 2017;39: 1010428317697558.
41. Li JZ, Zou K, Yu LH, Zhao WY, Lu Y, Mao J, Wang B, Wang L, Fan SJ, Song B, Li LH. MicroRNA-140 Inhibits the Epithelial-Mesenchymal Transition and Metastasis in Colorectal Cancer. Mol Ther-Nucl Acids. 2018;10:426-37.
42. Ortega FJ, Mercader JM, Moreno-Navarrete JM, Rovira O, Guerra E, Esteve E, Xifra G, Martinez C, Ricart W, Rieusset J, Rome S, Karczewska-Kupczewska M, Straczkowski M, Fernandez-Real JM. Profiling of Circulating MicroRNAs Reveals Common MicroRNAs Linked to Type 2 Diabetes That Change With Insulin Sensitization. Diabetes Care. 2014;37:1375-83.

Figures

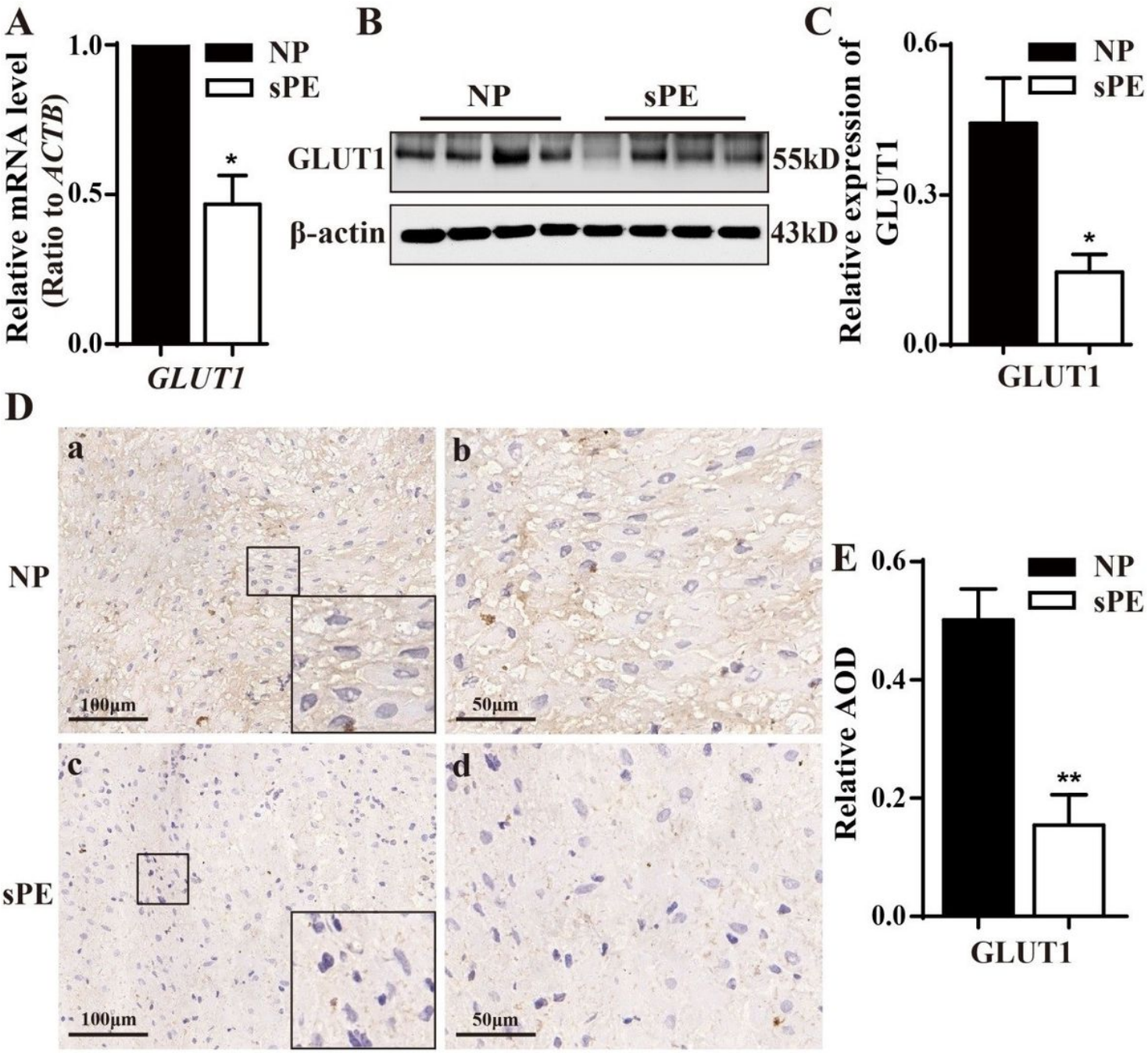

Figure 1

The expression of GLUT1 in decidual tissues from normal pregnant (NP) women and severe preeclampsia (sPE) patients. (A) Quantitative PCR analysis of GLUT1 mRNA level in decidual tissues from normal pregnancies (n=20) and sPE pregnancies (n=20). (B, C) Western blotting and densitometric analysis of the protein level of GLUT1 in decidual tissues from normal pregnancies and sPE pregnancies. (D) Immunohistochemical assay of GLUT1 in the decidual tissues of NP (a, b) and sPE woman (c, d). The inserts show enlarged immunolabelled decidual cells. NP: normal pregnant; sPE: severe preeclampsia. (E) The immunohistochemical results of decidual specimens from 12 NP and 12 sPE women (each woman 3 fields) were quantitatively analyzed and the data are exhibited as bar graph. AOD: Average optical density

(Integrated Optical Density/area). The data were expressed as mean ± SEM of three independent experiments. *, P < 0.05.

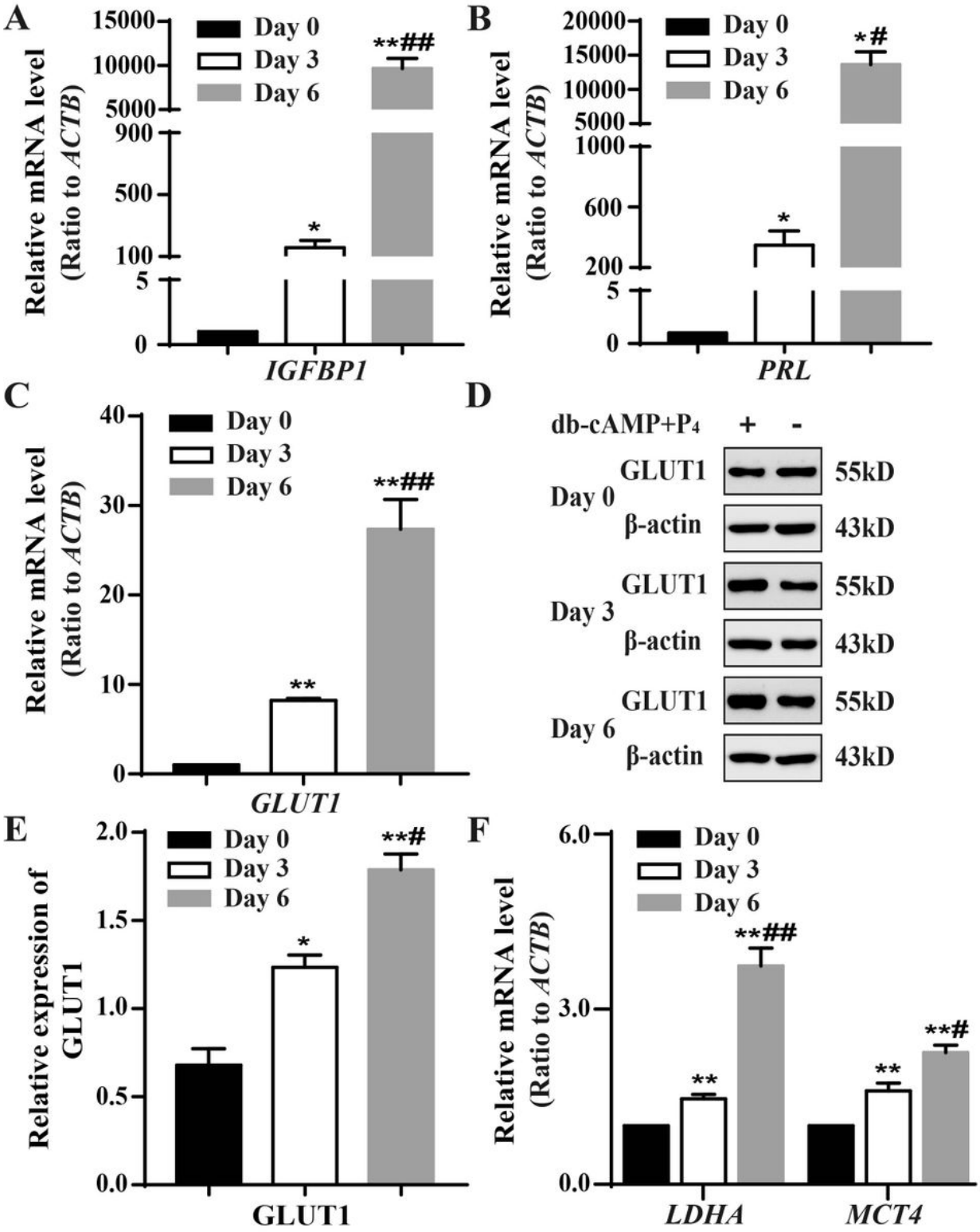

Figure 2

The expression of decidualization markers IGFBP1, PRL and GLUT1, LDHA and MCT4 in human endometrial stromal cells (hESCs) during in vitro decidualization. (A-C) The mRNA levels of IGFBP1, PRL and GLUT1 during in vitro decidualization. (D) Representative blots of GLUT1 and β-actin. (E) Statistical

analysis of the relative expression of GLUT1 protein to β-actin. (F) The mRNA levels of LDHA and MCT4 during in vitro decidualization. The data were expressed as mean ± SEM of three independent experiments. *, P < 0.05; **, P < 0.01 vs Day 0; #, P < 0.05; ##, P < 0.01 vs Day 3.

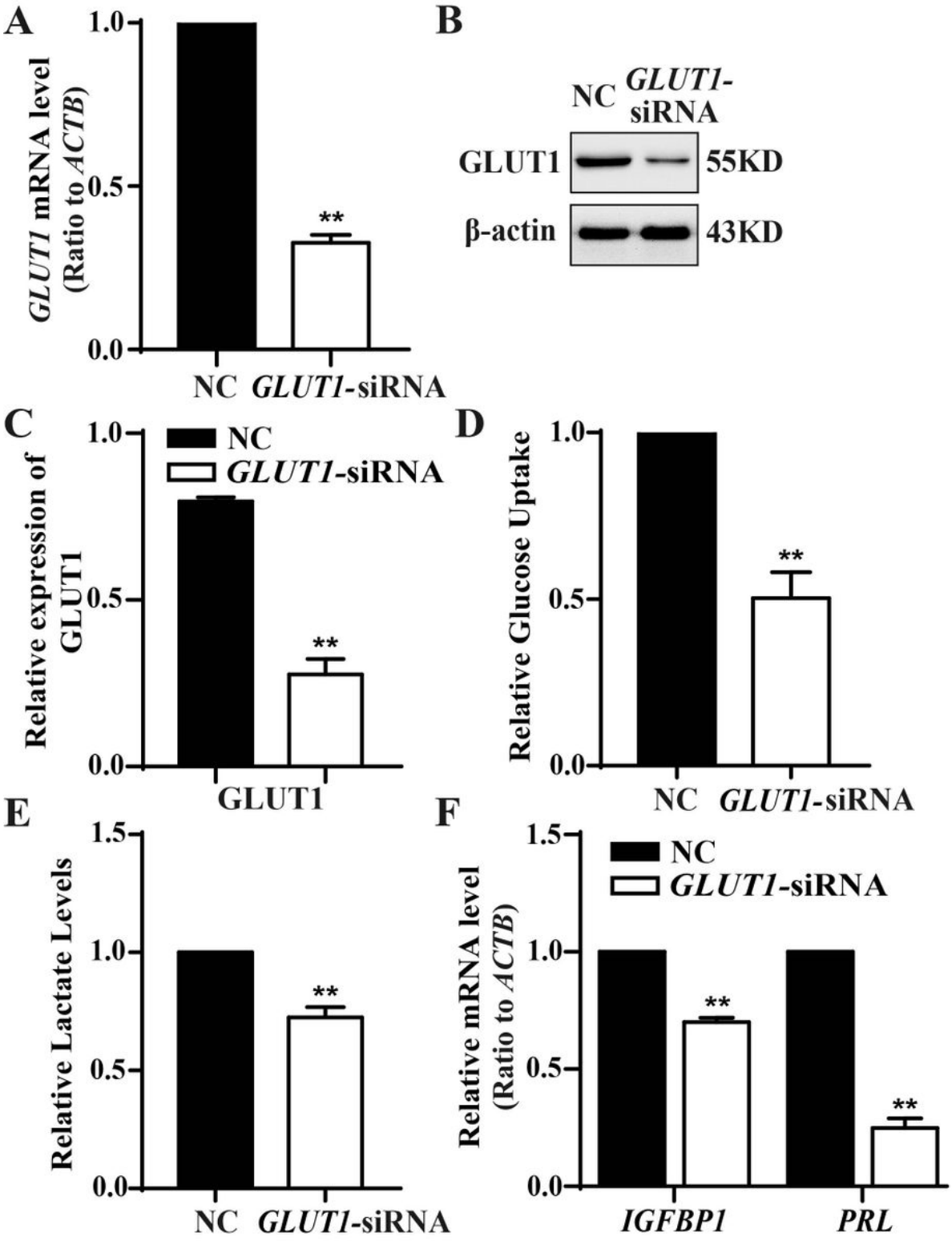

Figure 3

The effects of GLUT1 silencing on glycolysis and decidualization of human endometrial stromal cells (hESCs). (A) The mRNA level of GLUT1 after GLUT1 interference. (B) Representative blots of GLUT1 and

β-actin. (C) Statistical analysis of the relative expression of GLUT1 protein to β-actin. (D, E) Analysis of glucose uptake and lactate production in hESCs after GLUT1-siRNA transfection. (F) The mRNA levels of IGFBP1 and PRL when GLUT1 was silenced. The data were expressed as mean ± SEM of three independent experiments. NC indicates negative control. *, P < 0.05; **, P < 0.01.

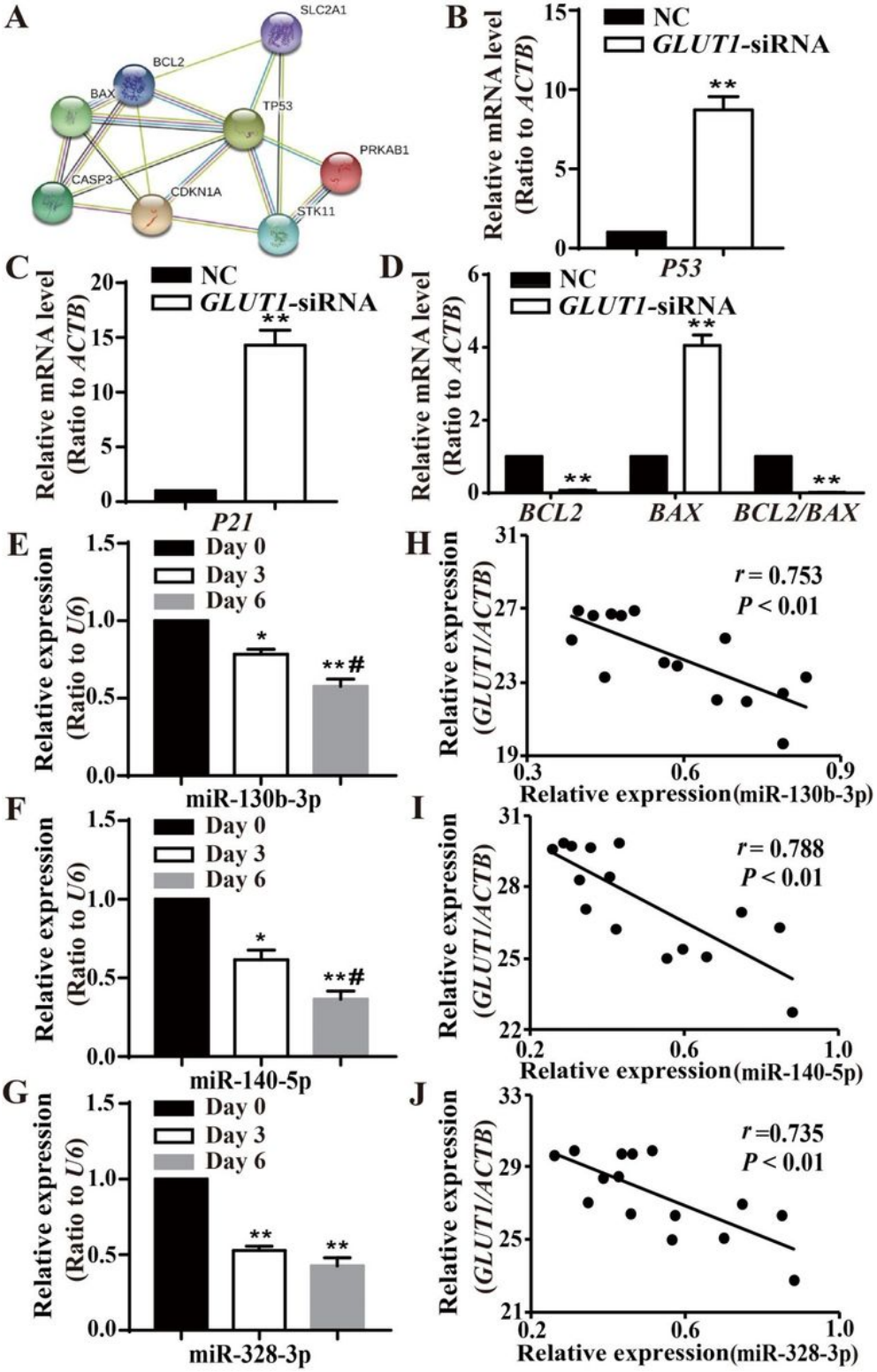

Figure 4

The activation of apoptotic pathways in human endometrial stromal cells (hESCs) in response to GLUT1 knockdown and the correlation analysis between miRNAs and GLUT1. (A) The interaction network predicted by STRING database showed proteins interacted with GLUT1. In the interaction network, each colored node represents the protein produced by a certain gene; edges indicate protein-protein associations. Different colors of lines show protein-protein associations from different sources. For example, purple line depicts the association between proteins as experimentally determined; yellow line indicates the association derives from databases and literatures. CDKN1A: P21; TP53: P53. (B) The mRNA level of P53 when GLUT1 was silenced. (C) The mRNA level of P21 when GLUT1 was silenced. (D) The mRNA level of BCL2 and BAX after GLUT1 knockdown. Subsequently, BCL2/BAX was calculated to evaluate the impact of GLUT1 knockdown on apoptosis. **, $P < 0.01$. (E-G) The levels of miR-130b-3p, miR-140-5p and miR-328-3p in hESCs treated with db-cAMP and P4 for 0, 3, 6 days respectively. The levels of miRNAs were adjusted by that of U6. (H-J) Analysis of the correlation of miR-130b-3p, miR-140-5p, miR-328-3p and GLUT1 mRNA levels in hESCs. Statistical analysis was performed using Pearson correlation analysis. The data were expressed as mean ± SEM of three independent experiments. NC indicates negative control. *, $P < 0.05$; **, $P < 0.01$ vs Day 0; #, $P < 0.05$ vs Day 3.

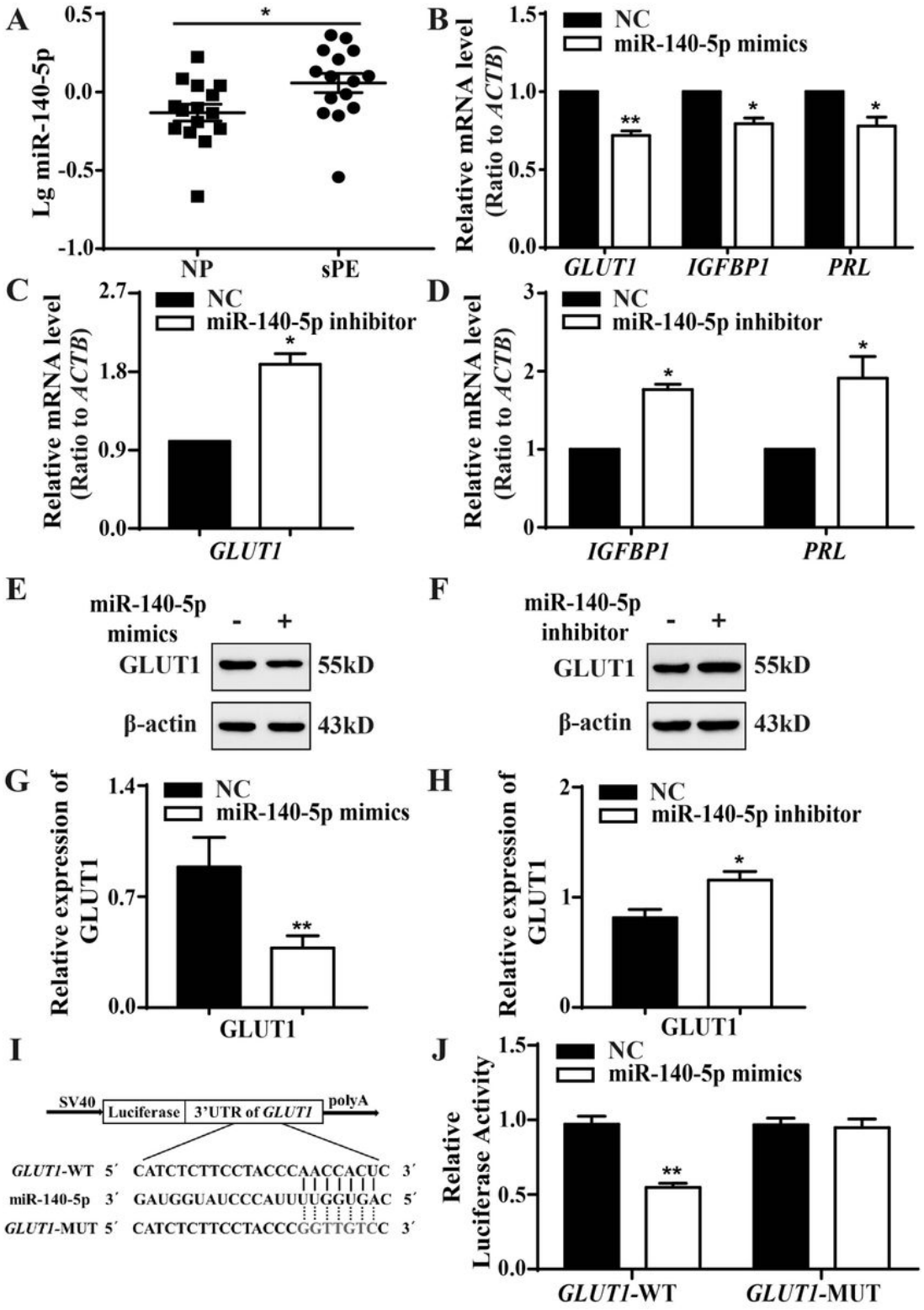

Figure 5

Validation of GLUT1 as a target gene of miR-140-5p in human endometrial stromal cells (hESCs). (A) The expression of miR-140-5p (log transformed) in severe preeclampsia (sPE) decidua (normal pregnant [NP] vs [sPE], 15 vs 15). (B) Quantitative PCR to show the mRNA levels of GLUT1, IGFBP1 and PRL in hESCs transfected with miR-140-5p mimics. (C, D) Quantitative PCR to show the mRNA levels of GLUT1, IGFBP1 and PRL in hESCs transfected with miR-140-5p inhibitor. (E-H) Western blotting to show GLUT1 protein

levels in hESCs transfected with miR-140-5p mimics and inhibitor. (I) Schematic map of the luciferase constructs. The construct containing the 3'-UTR segment of human GLUT1 is shown as wild type (WT), and mutant construct is shown as GLUT1-MUT (dotted lines, mutated binding sites). (J) Luciferase assay in hESCs co-transfected with the GLUT1-WT or GLUT1-MUT constructs and miR-140-5p or NC. The values presented were mean ± SEM of three independent experiments. NC indicates negative control. *, P < 0.05; **, P < 0.01.

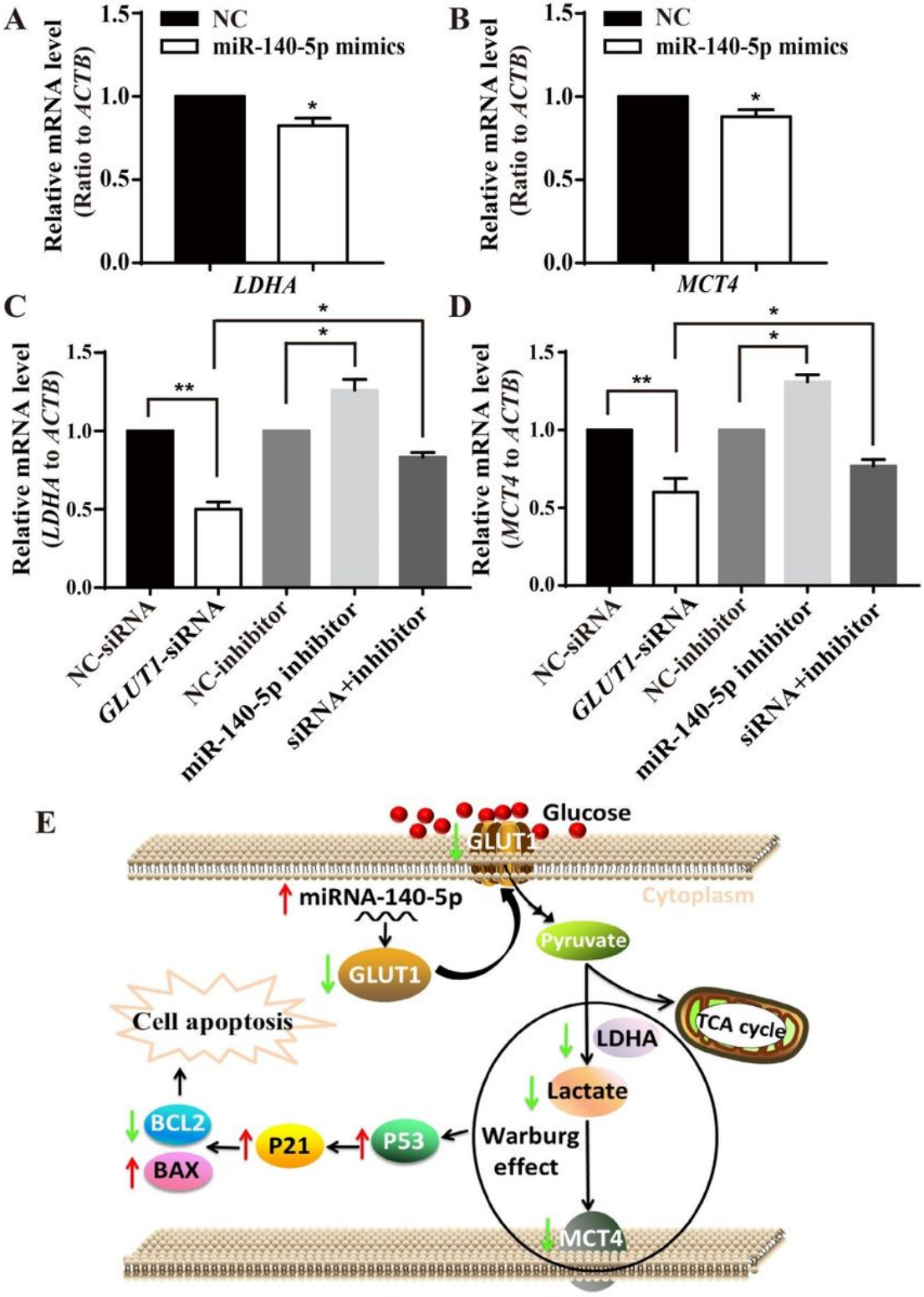

Figure 6

The effects of miR-140-5p on LDHA and MCT4 levels in human endometrial stromal cells (hESCs). (A, B) Quantitative PCR to show LDHA and MCT4 mRNA levels after miR-140b-5p mimics transfection. (C, D) Quantitative PCR to examine LDHA and MCT4 mRNA levels in hESCs transfected with NC-siRNA, GLUT1-siRNA, NC-inhibitor, miR-140-5p inhibitor or siRNA+inhibitor. The relative values were presented as mean ± SEM of three independent experiments. *, $P < 0.05$; **, $P < 0.01$. (E) A proposed schematic diagram uncovered the role of GLUT1 in the pathogenesis of PE. Reduced GLUT1 attenuated LDHA and MCT4, thus inhibiting the Warburg effect (aerobic glycolysis) in hESCs. Subsequently, apoptotic pathway factors P53, P21 and BAX were activated and BCL2 was inhibited, which impeded the decidualization and then led to PE. In this process, miR-140-5p acts as an upstream of GLUT1 to negatively regulate decidualization.

Supplementary Files

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