Glucose regulates the histone acetylation of gene promoters in decidualizing stromal cells

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Abstract

Decidualization stimuli activate the insulin signaling pathway and increase the glucose uptake in human endometrial stromal cells (ESCs). The inductions of prolactin (*PRL*) and IGF-binding protein-1 (*IGFBP1*), specific markers of decidualization, were inhibited by incubating ESCs under low glucose concentrations. These results suggested that decidualization stimuli activate the insulin signaling pathway, which contributes to decidualization through the increase of glucose uptake. Here, we investigated the mechanisms by which glucose regulates decidualization. ESCs were incubated with cAMP to induce decidualization. We examined whether low glucose affects the expression levels of transcription factors that induce decidualization. Forkhead box O1 (*FOXO1*) expression was significantly suppressed under low glucose conditions. Knockdown of *FOXO1* by siRNA inhibited the expression levels of *PRL* and *IGFBP1* during decidualization. Taken together, our results showed that low glucose inhibits decidualization by decreasing *FOXO1* expression. We also examined the levels of histone H3K27 acetylation (H3K27ac), which is related to active transcription, of the promoter regions of *FOXO1*, *PRL* and *IGFBP1* by ChIP assay. The H3K27ac levels of these promoter regions were increased by decidualization under normal glucose conditions, but not under low glucose conditions. Thus, our results show that glucose is indispensable for decidualization by activating the histone modification status of the promoters of *PRL*, *IGFBP1* and *FOXO1*.

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Introduction

Human endometrial stromal cells (ESCs) undergo cyclic changes by steroid hormones during the menstrual cycle. One of these changes is decidualization, which is crucial for embryo implantation and maintenance of pregnancy (Gellersen & Brosens 2003, Zhang & Yan 2016). Impaired decidualization of endometrial stroma is associated with recurrent miscarriage, implantation failure and unexplained infertility (Laird *et al.* 2006, Salker *et al.* 2010). A number of genes are upregulated or downregulated in ESCs undergoing decidualization (Popovici *et al.* 2000, Tamura *et al.* 2014a). Among these genes, IGF-binding protein-1 (*IGFBP1*) and prolactin (*PRL*) are induced in ESCs during decidualization and are therefore recognized as markers of decidualization (Gellersen & Brosens 2003, Tamura *et al.* 2012, Sultana *et al.* 2017).

By using a genome-wide RNA-sequence approach, we previously found that the insulin signaling pathway is selectively activated during decidualization in human ESCs (Tamura *et al.* 2014a). In ESCs during decidualization, we found increases in the expression of six insulin-related genes: forkhead box O1 (*FOXO1*), insulin receptor substrate 1 (*IRS1*), insulin receptor substrate 2 (*IRS2*), insulin receptor (*INSR*), mitogen-activated protein kinase

10 (*MAPK10*) and v-akt murine thymoma viral oncogene homolog 3 (*AKT3*). Each of these genes is reported to be involved in insulin actions, especially glucose uptake (Miura *et al.* 2004, Thirone *et al.* 2006). In fact, glucose uptake is increased by decidualization with the induction of *PRL* and *IGFBP1* (Tamura *et al.* 2014a). On the other hand, the increase of *PRL* or *IGFBP1* induced by decidualization stimuli was inhibited by incubating ESCs under low glucose conditions (Frolova & Moley 2011, Tamura *et al.* 2014a). These results suggest that decidualization stimuli activate the insulin signaling pathway, which in turn contributes to decidualization through the increase of glucose uptake. However, it is unclear how glucose is involved in decidualization in human ESCs.

Gene expression including transcription involves a change of chromatin structure, which can be regulated by epigenetic mechanisms such as histone modifications (Li *et al.* 2007, Tamura *et al.* 2011). Acetylation of histone-H3 lysine-27 (H3K27ac) is one of the active histone modifications and is highly enriched at the active promoter or enhancer regions (Wang *et al.* 2008). Glucose taken into a cell is metabolized to acetyl CoA, which serves as a source of acetyl modification of proteins, including histone proteins (Lu & Thompson 2012). This indicates

a close relationship between cellular glucose levels and epigenetic gene regulation. By using a genome-wide ChIP-sequence approach, we identified a number of upregulated genes in which the H3K27ac level was increased during decidualization in human ESCs (Tamura *et al.* 2014a, 2018), which indicates that an epigenetic mechanism plays an important role in the regulation of decidualization in human ESCs. We also reported that the H3K27ac level was increased by decidualization stimulus in the promoter regions of *PRL* and *IGFBP1*, which contributes to the induction of their gene expressions during decidualization (Tamura *et al.* 2014b). These facts led us to hypothesize that glucose is a critical factor regulating the expressions of decidualization-related genes through the modification of the H3K27ac levels of promoters.

In this study, we investigated the molecular mechanisms by which glucose regulates the expression of *PRL* and *IGFBP1* in human ESCs undergoing decidualization. We first focused on a transcription factor FOXO1, which regulates the expression of *PRL* and *IGFBP1* during decidualization, and found that glucose regulates FOXO1 expression by increasing the H3K27ac level of the promoter region. We further revealed that glucose also contributes to increase the H3K27ac level of the promoter region of *PRL* and *IGFBP1* during decidualization.

Materials and methods

Reagents

DMEM, L-glutamine, 1×trypsin-EDTA and antibiotic-antimycotic were obtained from Thermo Fisher Scientific. Fetal bovine serum (FBS) was obtained from Biological Industries Ltd (Beit Haemek, Israel). Collagenases, estradiol (E), medroxyprogesterone acetate (MPA) and dibutyl-*c*-AMP (db-*c*-AMP) were obtained from Sigma Chemical Co Ltd. Tissue flasks were from Becton Dickinson Co Ltd.

ESCs isolation and cell culture

Informed written consent was obtained from all participating patients, and ethical approval was obtained from Institutional Review Board of Yamaguchi University Hospital. All experiments were performed in accordance with Tenets of the Declaration of Helsinki.

Human endometrial tissues were obtained at hysterectomy from patients with a normal menstrual cycle, aged 40–45 years, who underwent surgery for myoma uteri or early stage of cervical cancer. The patients were not on hormonal therapy at the time of surgery. Endometrial samples utilized for ESCs isolation were histologically diagnosed as being in the late proliferative phase according to published criteria (Noyes *et al.* 1975). Tissue samples were washed with Phenol Red-free DMEM containing 4 mM glutamine, 50 µg/mL streptomycin and 50 IU/mL penicillin and minced into pieces of <1 mm³. ESCs were isolated as reported previously (Tamura *et al.* 2011). In brief, tissues were minced, enzymatically digested with 0.2% collagenase in a shaking water bath for 2 h at 37°C and filtered through a 70 µm nylon mesh. Stromal cells in the filtrates

were washed three times with the medium, and the number of viable cells was counted by Trypan blue dye exclusion. Under the microscope, all the cells reacted with the stromal-reacting antibody vimentin, indicating that they were homogeneous. The cells were also verified to be negative for the epithelial cell-reacting antibody (cytokeratin). Cells were seeded at 10⁵ cells/cm² in 75 cm² tissue culture flasks and incubated in phenol red-free DMEM containing glutamine, antibiotics and 10% dextran-coated charcoal-stripped FBS at 37°C, 95% air and 5% CO₂. At confluence, cells were treated with 1×trypsin-EDTA and subcultured into 25 cm² tissue culture flasks. At 80% confluence after the first passage, the cell culture medium was changed to the treatment medium. To induce decidualization, ESCs were incubated with treatment medium (phenol red-free DMEM supplemented with glutamine, antibiotics and 2% dextran-coated charcoal-stripped FBS) containing db-*c*-AMP (0.5 mM) for 4 days or E (10⁻⁸ mol/L) and MPA (10⁻⁶ mol/L) for 14 days at 37°C in an atmosphere of 95% air and 5% CO₂ as reported previously (Okada *et al.* 2018). The cells were then used for the following experiments described below. The concentrations of *c*-AMP and ovarian steroids and the period of incubation used in this study were based on our previous report (Matsuoka *et al.* 2010). The medium was changed every other day. To examine the importance of glucose for decidualization, ESCs were cultured in the medium containing normal (24 mM) or low (0 mM) glucose concentrations, which was based on previous reports (Frolova & Moley 2011, Tamura *et al.* 2014a), in the presence or absence of *c*-AMP for 4 days or E and MPA for 14 days. Decidualization was evaluated by mRNA expression of *PRL* and *IGFBP1*. For each experiment, a single incubation was performed in triplicate on cells isolated from one patient. Three different incubations from three individuals were performed in a single experiment.

Chromatin immunoprecipitation (ChIP)-quantitative PCR

H3K27ac levels of the promoter regions were examined by ChIP assay according to the protocol for the ChIP assay kit (Upstate Biotechnology, Lake Placid, NY, USA) as reported previously with some modifications (Lee *et al.* 2013, Tamura *et al.* 2014b). Cells were cross-linked by the addition of formaldehyde into the medium at a final concentration of 1% and incubated for 10 min at 37°C. Cross-linking was terminated by addition of glycine (0.125 M, final concentration). Cells were washed with ice-cold PBS containing protease inhibitors (Sigma) and resuspended in ChIP lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0, with protease inhibitors). The lysates were sonicated using a Bioruptor ultrasonicator (Cosmo-bio, Tokyo, Japan), precleared with salmon sperm DNA-protein A at 4°C for 4 h and diluted with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.0, 167 mM NaCl, with protease inhibitors). Five percent of the supernatants were kept as input controls (INPUT). Dynabeads Protein A (Invitrogen) were incubated with antibodies for H3K27ac (generous gifts from Dr Kimura) (Kimura *et al.* 2008) and normal mouse IgG (Invitrogen) 4°C overnight. The precleared chromatin was incubated with antibody-bound Dynabeads for 6 h at 4°C. Immune complexes were collected and washed once for 5 min on a rotating platform with 1 mL each of the following buffers in sequence: low salt wash buffer (0.1% SDS, 1% Triton X-100,

2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 1500 mM NaCl), LiCl wash buffer (250 mM LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0), and twice with TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Immune complexes were eluted with 200 μ L elution buffer (1% SDS, 0.1 M NaHCO₃, 10 mM DTT). The immunoprecipitated chromatin complexes (IP) and INPUT were incubated at 65°C overnight to reverse the cross-linking and subjected to proteinase K treatment. Purified DNA using a QIAquick PCR purification kit (QIAGEN) served as the template for PCR. ChIP primers were designed in the promoter region of PRL, IGFBP1 and FOXO1 as we reported previously (Tamura *et al.* 2014a, 2017) (Supplementary Table 1, see section on [supplementary data](#) given at the end of this article). Real-time PCR was used to determine the relative levels of H3K27ac in the promoter regions of PRL, IGFBP1 and FOXO1. The ratio of IP DNA to the INPUT DNA sample (%INPUT) was calculated.

Total RNA extraction and real-time RT-PCR

Total RNA was isolated from the cultured cells with an RNeasy® Mini Kit (QIAGEN). The RNA was reverse transcribed as reported previously (Maekawa *et al.* 2016). For PCR amplification, first-strand cDNA was synthesized from 1 μ g total RNA with reverse transcriptase (Invitrogen) in 20 μ L of reaction mixture. Real-time RT-PCR was performed as reported previously (Okada *et al.* 2016) using sequence-specific primer sets (Supplementary Table 1).

Glucose uptake assay

The levels of glucose uptake in ESCs were determined using a 2-deoxyglucose (DG) uptake assay kit (Cosmobio) as reported previously (Tamura *et al.* 2014a). Cells were incubated with serum and glucose free-DMEM for 6 h. Cells were washed three times with Krebs-Ringer-phosphate-Hepes (KRPH) buffer (20 mM Hepes, 5 mM KH₂PO₄, 1 mM MgSO₄, 1 mM CaCl₂, 136 mM NaCl and 4.7 mM KCl, pH 7.4, 37°C) containing 2% bovine serum albumin (BSA) and were further incubated at 37°C for 20 min with KRPH buffer containing 2% BSA and 0.1 mM 2-DG. Then, the cells were washed three times with PBS, collected in 400 μ L of 10 mM Tris-HCl (pH 8.0), heated at 80°C for 15 min and centrifuged at 15,000g for 20 min at 4°C. The supernatants were assayed for a 2-DG uptake measurement. A single incubation was performed in triplicate on cells isolated from one patient. Three different incubations from three individuals were performed in a single experiment.

Western blotting

Whole cell lysates were prepared using loading buffer reagents (New England Biolabs, Herts, UK) without trypsin treatment. Equal amounts of total protein were electrophoresed on a 10% SDS-PAGE. The proteins were transferred to PVDF membranes (ATTO, Tokyo, Japan). The membranes were blocked with blocking solution (5% skimmed milk with 0.1% Tween-20 dissolved in Tris buffered saline (pH 7.5)), incubated with the first antibody for FOXO1 (Santa Cruz Biotechnology, Inc.), p-S473 AKT (Cell Signaling Technology) and β -tubulin (Sigma), which were diluted in blocking solution, incubated with the

peroxidase-conjugated second antibody diluted in blocking solution, visualized with the ECL Prime Western blotting detection system (Amersham, Aylesburg, UK) according to the manufacturer's protocol and used to expose hyperfilm-ECL (Amersham). To reuse the blot, the membranes were stripped in Restore Western stripping buffer (Pierce).

Transfection of siRNA

FOXO1 ON-TARGET plus SMART pool siRNA and ON-TARGET plus Non-Targeting pool siRNA were purchased from Dharmacon (Lafayette, CO, USA). siRNA was transfected to ESCs as reported previously (Tamura *et al.* 2017). In brief, ESCs were plated in the medium lacking antibiotics and incubated for 2 days. At 50–60% confluence, siRNA duplexes (50 nM) and RNAi MAX (Invitrogen) diluted in OptiMEM (Invitrogen) were transfected to ESCs. The medium was changed to DMEM containing 10% FBS 5 h later. After 48 h of transfection, cells were incubated in the presence or absence of cAMP for 4 days.

Statistical analysis

Statistical significance was determined by one-way ANOVA. After ANOVA, the Tukey–Kramer test was applied to analyze the differences between groups. An unpaired *t* test was applied to analyze the differences between two groups. All statistical analyses were performed using SPSS for Windows version 11 (SPSS Inc.). Differences were considered significant at *P* < 0.05.

Results

Effects of cAMP on the expression of insulin signaling-related genes and glucose uptake in ESCs

We previously reported that the expression of insulin signaling-related genes and the glucose uptake in ESCs were increased by decidualization that was induced by E+MPA stimulation (Tamura *et al.* 2014a). In this study, to focus on the molecular mechanism by which glucose contributes to decidualization, we used cAMP alone as a simple decidualization stimulus as reported previously (Gellersen & Brosens 2003, Tamura *et al.* 2014b, 2017, Liang *et al.* 2016). We first examined whether cAMP also increases the expression of insulin signaling-related genes. Six genes (FOXO1, IRS1, IRS2, INSR, MAPK10 and AKT3) were selected as the target genes because E+MPA stimulation increased these gene expressions (Tamura *et al.* 2014a). cAMP significantly increased these gene expressions (Fig. 1A). Glucose uptake was also significantly increased by cAMP stimulation in ESCs (Fig. 1B). These results indicated that cAMP stimulation as well as E+MPA stimulation activates the insulin signaling pathway and increases glucose uptake in human ESCs.

Effects of low glucose on the expression of PRL and IGFBP1 by decidualization

To investigate the role of glucose in decidualization, ESCs were incubated under the environment of

normal glucose concentration (24 mM) or low glucose concentration (0 mM) in the presence or absence of cAMP, and then decidualization was evaluated by mRNA expression of *PRL* and *IGFBP1*. mRNA levels of *PRL* and *IGFBP1* were remarkably increased by cAMP under normal glucose conditions, and these increases were significantly inhibited under low glucose conditions (Fig. 2). Low glucose conditions did not affect the morphological changes of ESCs induced by cAMP (data not shown). These findings were also confirmed in decidualization induced by E+MPA (Supplementary Fig. 1) (Tamura et al. 2014a). These results indicate that glucose contributes to the gene expressions of *PRL* and *IGFBP1* in ESCs undergoing decidualization.

Effects of low glucose on the expressions of decidualization-related transcription factors

We hypothesized that the expression levels of transcription factors that are involved in the regulation of *PRL* and *IGFBP1* during decidualization are suppressed by low glucose conditions. To test this hypothesis, we examined the effect of low glucose on the mRNA expressions of three transcription factors that are involved in the regulation of *PRL* and *IGFBP1* during decidualization:

Figure 1 Effects of cAMP on the expression of insulin signaling-related genes and glucose uptake in ESCs. ESCs were treated with or without cAMP for 4 days to induce decidualization. Cells treated without cAMP were used as the control. (A) Relative mRNA levels of insulin signaling-related genes were quantified by real-time RT-PCR. mRNA levels were normalized to those of *GAPDH* and expressed as a ratio of the control ESCs. Values are mean $\pm$ s.d. of three different incubations. ^a $P < 0.05$ vs control. (B) Effect of cAMP on glucose uptake, as expressed by a 2-DG uptake assay. Glucose (2-DG) uptake was expressed as a ratio of the control ESCs. Values are mean $\pm$ s.d. of three different incubations. ^a $P < 0.05$ vs control.

Figure 2 Effects of low glucose on the expression of *PRL* and *IGFBP1* by decidualization. ESCs were cultured in medium containing normal glucose concentration (24 mM) or low glucose concentration (0 mM) with or without cAMP. Relative mRNA levels of *PRL* and *IGFBP1* were quantified by real-time RT-PCR. mRNA levels were normalized to those of *GAPDH* and expressed as a ratio of the cAMP-treated sample under the normal glucose concentration (24 mM). ^a $P < 0.01$ vs control (24 mM); ^b $P < 0.01$ vs cAMP (24 mM).

FOXO1, Signal Transducer And Activator Of Transcription 5A (*STAT5A*) and CCAAT/enhancer-binding protein β (*C/EBP β*) (Mak et al. 2002, Kim et al. 2003, Takano et al. 2007, Nagashima et al. 2008, Lynch et al. 2009, Wang et al. 2012, Tamura et al. 2014b). As shown in Fig. 3A, *FOXO1* mRNA levels were remarkably increased by cAMP under normal glucose conditions, and this increase was partially blocked under low glucose conditions. On the other hand, mRNA levels of other two transcription factors (*STAT5A* and *C/EBP β*) were not affected by low glucose. These findings were also confirmed in decidualization induced by E+MPA under low glucose conditions (Supplementary Fig. 2). These results indicate that glucose is one of the factors contributing to *FOXO1* expression in ESCs undergoing decidualization.

AKT, also known as protein kinase B, is serine/threonine kinase that regulates decidualization (Fabi et al. 2017). It is well known that phosphorylation of a serine residue (S473) on AKT (p-S473 AKT) is inhibited during decidualization, which is important signaling for decidualization and increase the expression of *FOXO1* in decidualized ESCs (Labied et al. 2006, Yin et al. 2012). Therefore, we examined whether low glucose conditions affect the expression levels of p-S473 AKT. As shown in Fig. 3B, cAMP decreased the expression levels of p-S473 AKT. Low glucose conditions did not affect it. These results show that the inhibitory effect of low glucose conditions on *FOXO1* expression is not mediated through the regulation of p-S473 AKT.

Involvement of FOXO1 in cAMP-induced gene expressions of PRL and IGFBP1

To examine the involvement of *FOXO1* in the expression of *PRL* and *IGFBP1*, ESCs were transfected with *FOXO1* siRNA to knockdown *FOXO1*. *FOXO1* protein expression was knocked down by *FOXO1* siRNA

Figure 3 Effects of low glucose on the expressions of decidualization-related transcription factors and the phosphorylation of AKT. (A) ESCs were cultured in medium containing normal glucose concentration (24 mM) or low glucose concentration (0 mM) with or without cAMP. Relative mRNA levels of *FOXO1*, *STAT5A* and *C/EBPβ* were quantified by real-time RT-PCR. mRNA levels were normalized to those of *GAPDH* and expressed as a ratio of the cAMP-treated sample under the normal glucose concentration (24 mM). ^a $P < 0.01$ vs control (24 mM); ^b $P < 0.01$ vs cAMP (24 mM); ^c $P < 0.01$ vs control (0 mM). (B) ESCs were cultured in medium containing normal glucose concentration (24 mM) or low glucose concentration (0 mM) with or without cAMP. Whole-cell lysates were prepared and subjected to Western blotting to examine the expression levels of p-S473 AKT. β-tubulin was used as an internal control. The immunoblot is a representative of three different incubations.

(Fig. 4A). The cAMP-induced increases in the mRNA levels of *PRL* and *IGFBP1* were significantly inhibited by FOXO1 siRNA (Fig. 4B). Knockdown of FOXO1 did not affect the morphological changes of ESCs induced by cAMP (Fig. 4C).

Effects of low glucose on H3K27ac levels of the promoter regions of FOXO1, PRL and IGFBP1

Epigenetic regulation including histone modification plays key roles in gene expressions during decidualization (Tamura *et al.* 2014a,b). In addition, low glucose environment has the potential to change the histone acetylation status of gene promoters (Wellen *et al.* 2009). Therefore, we examined whether low glucose conditions affect the H3K27ac levels of the FOXO1 promoter region during decidualization. Under normal glucose conditions, cAMP remarkably increased the H3K27ac level of the FOXO1 promoter region, but not under low glucose conditions (Fig. 5). These results suggest that low glucose conditions suppress FOXO1 expression by decreasing the H3K27ac levels of the promoter region.

We reported that decidualization stimuli increase the H3K27ac levels of the promoter region of *PRL* and *IGFBP1* (Tamura *et al.* 2014b), which is necessary for the induction of their gene expressions. Therefore, we examined whether low glucose conditions also affect the increase of the H3K27ac levels of the promoter region of *PRL* and *IGFBP1* by decidualization. The H3K27ac

Figure 4 Involvement of FOXO1 in cAMP-induced gene expressions of *PRL* and *IGFBP1*. (A) ESCs were transfected with a siRNA targeted against FOXO1 or with a non-targeting siRNA as a control. 48 h after siRNA transfection, ESCs were treated with or without cAMP for 4 days. Whole-cell lysates were prepared and subjected to Western blotting to confirm the knockdown of FOXO1. β-tubulin was used as an internal control. The immunoblot is a representative of three different incubations. (B) Relative mRNA levels of *PRL* and *IGFBP1* were quantified by real-time RT-PCR. mRNA levels were normalized to those of *GAPDH* and expressed as a ratio of the cAMP treatment sample which was transfected with control siRNA. Values are mean $\pm$ s.d. of three different incubations. ^a $P < 0.01$ vs control treatment; ^b $P < 0.01$ vs cAMP treatment in the control siRNA; ^c $P < 0.01$ vs control treatment. (C) Morphologies of non-treated (control) or cAMP-treated ESCs with the knockdown of FOXO1.

levels of the *PRL* and *IGFBP1* promoter regions were remarkably increased by cAMP under normal glucose conditions, and these increases were inhibited under low glucose conditions. These results show that low glucose conditions decrease the H3K27ac levels not only in the FOXO1 promoter region but also in the *PRL* and *IGFBP1* promoter regions.

We further examined whether FOXO1 overexpression recovers the decreases in *PRL* and *IGFBP1* mRNA levels caused by low glucose conditions. For this purpose, ESCs were infected with retrovirus encoding FOXO1 to overexpress FOXO1 and incubated under the environment of normal glucose conditions (24 mM) or low glucose conditions (0 mM) in the presence or absence of cAMP. FOXO1 overexpression increased

Figure 5 Effects of low glucose on H3K27ac levels of the promoter regions of *FOXO1*, *PRL* and *IGFBP1*. ESCs were cultured in medium containing normal glucose concentration (24 mM) or low glucose concentration (0 mM) with or without cAMP. H3K27ac levels in the promoter region of *FOXO1*, *PRL* and *IGFBP1* were analyzed by ChIP assay. Normal mouse IgG was used as a negative control. The H3K27ac levels were analyzed by real-time PCR. Data were plotted as the ratio of IP DNA to the total INPUT DNA sample (%INPUT). Values are mean $\pm$ s.d. of three different incubations. ^a $P < 0.05$ vs control (24 mM); ^b $P < 0.05$ vs cAMP (24 mM). All PCR products from INPUT or IP DNA were electrophoresed on the same gel, and the representative ethidium bromide-stained gels are shown.

mRNA levels of *PRL* and *IGFBP1* under low glucose conditions (data not shown), but it seemed to act in a different manner from the glucose-driven upregulation of *PRL* and *IGFBP1* rather than that *FOXO1* rescued the decreases in *PRL* and *IGFBP1* mRNA levels caused by low glucose. Therefore, it is still unclear to what extent *FOXO1* contributes to the glucose-driven upregulation of *PRL* and *IGFBP1*.

Discussion

We previously reported that the insulin signaling pathway is activated and glucose uptake is increased during decidualization in human ESCs. Glucose is necessary for decidualization of ESCs (Frolova & Moley 2011, Tamura et al. 2014a). However, the mechanism that regulates decidualization by glucose has not been well clarified. The present study demonstrated that glucose contributes to decidualization through an epigenetic mechanism.

Histone modifications, one of the epigenetic mechanisms, regulate gene expression by changing the chromatin structure of promoters or enhancer regions (Li et al. 2007, Tamura et al. 2011). Acetylation of H3K27 (H3K27ac) is one of the histone modifications, and H3K27ac of the promoter activates gene transcription (Creyghton et al. 2010). During decidualization of human ESCs, H3K27ac levels are increased in the promoter regions of *PRL* and *IGFBP1*, which contributes to the upregulation of *PRL* and *IGFBP1* expression (Tamura et al. 2014b).

The present study clearly showed that low-glucose environment during decidualization suppressed the H3K27ac levels of the promoter region of *FOXO1* and cAMP-increased *FOXO1* mRNA levels. *FOXO1* is an important transcription factor for decidualization and regulates a number of genes related to decidualization (Takano et al. 2007), such as *PRL* and *IGFBP1*. Therefore, a low-glucose environment inhibits *FOXO1* expression

by decreasing H3K27ac levels of the promoter, which in turn suppresses the gene expressions of *PRL* and *IGFBP1*.

Our results also showed that a low-glucose environment suppressed the H3K27ac levels of the *PRL* and *IGFBP1* promoter regions. Thus, low glucose environment suppresses the mRNA levels of *PRL* and *IGFBP1* during decidualization by not only inhibiting the expression levels of *FOXO1* but also by directly decreasing the H3K27ac levels of the promoter regions of *PRL* and *IGFBP1*. Taken together, our results show quite new findings that glucose contributes to decidualization through the epigenetic regulation in human ESCs.

Generally, acetylation of proteins starts with the uptake of glucose by the cells, which is then metabolized to acetyl CoA via glycolysis and TCA cycle. Acetyl CoA serves as a source of acetyl modification of proteins, including histone proteins (Lu & Thompson 2012). Therefore, a low glucose environment decreases the cellular levels of acetyl CoA (Wellen et al. 2009, Yang et al. 2015). This raises the possibility that low glucose conditions suppress H3K27ac levels and mRNA expression levels genome wide. However, not all gene expressions are affected by low glucose (Arumugam et al. 2011, Yang et al. 2015, Hien et al. 2016). This is consistent with our finding that the mRNA expression levels of *STAT5A* and *C/EBP β* were not affected by low glucose. It is likely that there are some gene-specific mechanisms that a low-glucose environment affects the H3K27ac level of the certain promoter regions. H3K27ac levels of the promoter regions are regulated by the recruitment of cofactors with histone acetyl transferase (HAT) activities. Because some of these cofactors are downregulated by low glucose, the genes that they regulate are suppressed by low glucose (Yang et al. 2015). Cofactors with HAT activities play an important role in the regulation of the expression of *PRL*, *IGFBP1* and *FOXO1* (Tamura et al. 2014b, Wondisford et al. 2014, Zhang et al. 2018). We thus speculate that low glucose inhibits the recruitment of cofactors with HAT activities to the promoter regions

of *PRL*, *IGFBP1* and *FOXO1* during decidualization. Further studies are needed to clarify the mechanisms by which low-glucose environment affects the H3K27ac levels in a gene-specific manner.

Polycystic ovary syndrome (PCOS) is an endocrine disorder of reproductive-aged women, which is characterized by anovulation and insulin resistance (Balén *et al.* 2016) and which causes problems in the endometrium such as implantation failure and ovarian dysfunction (Bellver *et al.* 2011). In fact, induction of *IGFBP1* expression by decidualization stimuli is impaired in ESCs from women with PCOS (Piltonen *et al.* 2015). The expressions levels of genes involved in glucose uptake, such as *INSR*, *IRS1* and glucose transporter type4 (*GLUT4*) are low in the endometrium with PCOS (Ujvari *et al.* 2014). The present results lead us to speculate that PCOS impairs glucose uptake in ESCs, which in turn would impair decidualization. This may be one of the reasons for implantation failure in PCOS.

In summary, our results show a new molecular mechanism by which glucose contributes to decidualization. Glucose is indispensable for decidualization because it activates the histone modification status of the promoters of *PRL*, *IGFBP1* and *FOXO1*, which in turn contributes to decidualization in ESCs. The insulin signaling pathway is activated during decidualization to increase glucose uptake. This phenomenon contributes to proper decidualization.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/REP-18-0393>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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