Regulation of insulin signaling and glucose transporters in the endometria of patients with polycystic ovary syndrome

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Regulation of insulin signaling and glucose transporters in the endometria of patients with polycystic ovary syndrome

Directed by Professor Byung Seok Lee

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This certifies that the Doctoral Dissertation of Mee-Hwa Lee is approved.

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Mee-Hwa Lee.

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ABSTRACT

Regulation of insulin signaling and glucose transporters in the endometria of patients with polycystic ovary syndrome

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Objective:

Subfertility associated with polycystic ovarian syndrome (PCOS) mainly originates from oligo/anovulation; however, factors related to blastocyst implantation and maintenance of pregnancy may also contribute. Although roles for insulin resistance and androgen excess have been suggested in the pathogenesis of PCOS-associated implantation failure, a comprehensive investigation of the consequences of PCOS on endometrial homeostasis and pathophysiology has not been performed. In this study, I investigated whether insulin sensitivity and glucose metabolism in the endometria of patients with PCOS are intrinsically altered. I also examined whether hyperandrogenic

milieu affect glucose metabolism and the global gene expression patterns in cultured human endometrial stromal cells (hESCs), and studied the influence of hyperandrogenic conditions on facilitated glucose transporters (GLUTs) expression during *in vitro* decidualization of hESCs.

Design:

Experimental study involved the use of human endometrial tissues and a human endometrial cell line.

Materials and Methods:

Seven healthy women with regular menstrual cycles and 16 patients with PCOS were recruited for this study. Endometrial samples were obtained from the corpus of the uteri under sterile conditions. Control endometria were biopsied in the early proliferative phase, due to morphological and physiological similarities to those of patients with PCOS. Reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, and western blot analysis were performed to examine the levels of proteins involved in insulin signaling and glucose metabolism. To mimic hyperandrogenism and hyperinsulinemia in patients with PCOS *in vitro*, hESCs were treated with 1, 10, or 100 μM dihydrotestosterone (DHT) for 3 to 9 days and 10 nM insulin for 8 hours, respectively. *In vitro* decidualization

was induced in hESCs by culture with 0.5 mM cAMP and 1 μM medroxyprogesterone 17-acetate for 1 to 9 days. Messenger RNA (mRNA) microarray experiments were performed with hESCs treated with 10 μM DHT, and data were analyzed using Gene Set Enrichment Analysis (GSEA).

Results:

In the endometria of patients with PCOS, GLUT1, GLUT12, insulin receptor (IR), and insulin receptor substrate (IRS) levels were significantly increased. However, no differences in expression were observed between the endometria of obese and lean patients with PCOS. Notably, GLUT4 mRNA was not detected in the endometria of patients with PCOS nor control subjects.

Levels of phosphorylated IRS1 (p-IRS1) and phosphorylated Akt (p-Akt) were up-regulated in the lysates of both insulin- and androgen-treated hESCs. In addition, DHT treatment up-regulated the levels of GLUT1 and GLUT12 in cultured hESCs, suggesting that hyperandrogenic conditions affect glucose transport and/or metabolism. This notion was supported by mRNA microarray experiments, which further demonstrated that glucose transport and/or metabolism was dysregulated in hESCs by DHT treatment.

During *in vitro* decidualization, the expression levels of GLUT1, 8, and 12 gradually increased. Of note, protein and mRNA levels of GLUT1 and 12 were decreased when decidualizing hESCs were treated with DHT, although these changes were not statistically significant.

Conclusion:

Hyperandrogenic milieu up-regulated the adaptor protein of insulin signaling as well as GLUTs in the endometria of patients with PCOS. Messenger RNA microarray experiments revealed androgen-induced dysregulation of glucose transport and/or metabolism in human endometrium. During decidualization, hyperandrogenic conditions down-regulated the expression of GLUTs, possibly causing impaired uterine receptivity.

Key words: endometrium, polycystic ovary syndrome, glucose transporter, androgen, decidualization

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I. INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most prevalent female endocrine conditions, which substantially compromises the health and reproductive capacity of women. Currently, the prevalence of PCOS ranges widely (from 4 to 21% in women of reproductive age), mainly due to the diagnostic criteria used, and partly due to differences in ethnicity as well as the approaches used to define PCOS phenotypes.[1] The pathophysiology of the classic PCOS phenotype is primarily characterized by an overexpression of steroidogenic enzymes, mostly cytochrome P450c17, in ovarian theca cells, resulting in functional ovarian hyperandrogenism (FOH).[2,3] Excessive luteinizing hormone (LH) stimulation as well as hyperinsulinemia are

considered co-factors in the hyperandrogenic milieu of PCOS[4]. Obesity and/or insulin resistance occur in one- to two-thirds of adult women with PCOS, contributing to their increased lifetime risk of developing type 2 diabetes mellitus (DM) and cardiovascular disease.[4-6]

Impaired reproductive capacity associated with PCOS mainly encompasses ovulatory dysfunction; however, factors related to blastocyst implantation and maintenance of pregnancy may also contribute.[7] More than 80% of women with recurrent miscarriages display polycystic ovarian morphology.[8] In addition, higher rates of miscarriage as well as decreased decidual endovascular trophoblast invasion were reported in patients with PCOS.[9,10] Recently, altered expression of genes related to implantation and unexplained infertility was demonstrated in the endometria of women with PCOS during the implantation window.[11] There is evidence of a role for insulin resistance in the pathogenesis of implantation failure and recurrent pregnancy loss with PCOS.[12,13] In addition, several *in vitro* studies have reported adverse effects of androgen on endometrial cell growth, as well as on endometrial decidualization and implantation[14,15] Despite these studies, the mechanisms underlying impaired endometrial receptivity in women with PCOS are still not completely understood.

The human endometrium exhibits steroid-dependent cyclic changes, requiring intensive glucose metabolism and GLUTs.[16] A quantitative evaluation of many GLUTs has recently been described in a non-PCOS uterus before and after decidualization[17]; nonetheless, a detailed and comprehensive quantification of GLUTs has not been performed in the endometria of patients with PCOS. In humans, there are 14 different GLUT proteins[18]; however, the majority of early studies have focused on the expression of GLUT4 and/or GLUT1 in the human endometrium. Reduced expression of GLUT4, an insulin-sensitive transporter, first suggested the occurrence of defective insulin signaling in the endometrium of patients with PCOS.[19-24] These findings formed the basis of the supposition that the uterine endometrium, like muscle and fat, is an insulin-sensitive tissue. However, other studies have failed to demonstrate detectable levels of GLUT4 in the human endometrium, particularly in endometrial stromal cells.[17,20]

Studies using hESCs revealed the critical role of GLUT1, a ubiquitous non-insulin-dependent glucose transporter, in decidualization and uterine receptivity.[16,17] GLUT1 expression significantly increases during ESC decidualization *in vitro*, and is up-regulated by progesterone (P_4).[25,26] These findings indicate that proper GLUT1 function is important for decidualization and glucose uptake in these tissues; however, no specific data evaluating the role of other GLUTs, nor the influence of hyperandrogenic conditions on the decidualization process and uterine receptivity,

are currently available. Notably, transcriptional profiling in a pathway-oriented analysis of endometrial cells from patients with PCOS has recently identified a variety of dysregulated signaling pathways.[27] In that study, hierarchical clustering revealed distinctly down-regulated gene sets, including those involved in the cell cycle, apoptosis, glycolysis, and the cytoskeletal network.

The first objective of this study was to perform a detailed and comprehensive quantification of a series of GLUTs in the endometria of patients with PCOS and to investigate whether insulin sensitivity and glucose metabolism were intrinsically altered in these patients. The second objective was to investigate whether hyperandrogenic conditions affect the levels of insulin-signaling proteins, GLUTs, and the expression patterns of specific gene sets, including those related to the cell cycle and glucose metabolism, in cultured hESCs. The final objective was to investigate the influence of hyperandrogenic milieu on GLUT expression during *in vitro* decidualization of hESCs.

II. MATERIALS AND METHODS

1. Ethics statement

All procedures used in this study were carried out in accordance with the principles for conducting experiments on human subjects outlined in the Declaration of Helsinki. The Institutional Review Board (IRB) of the CHA Bundang Medical Center of CHA University, Gyeonggi-do, Korea (IRB No BD2011-115D), approved this study. All human subjects signed a written informed consent form before participating in the study.

2. Subjects

Subjects were enrolled between July 2013 and November 2014. Study subjects included 16 patients with PCOS and 7 normally cycling women. The diagnosis of PCOS was based on the presence of at least two of the criteria suggested by the revised 2003 Rotterdam consensus for PCOS, i.e. oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries.[5] Control subjects had regular menstrual cycles (at 25 to 35 day intervals) with no clinical or biochemical signs of PCOS. Additional inclusion criteria for all subjects were an age of 18–40 years, and no hormonal treatments, including oral contraceptives, for at least 3 months before entering the study. In addition, no subjects presented with

thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia, Cushing syndrome, or androgen-producing tumors.

3. Clinical and biochemical assessments

Clinical assessment comprised anthropometric measurements, modified Ferriman–Gallwey score calculation, and blood pressure determination. Subjects were asked to answer a questionnaire regarding their family history of hypertension, DM, and other heritable endocrine conditions.

To assess basal hormone levels, venous blood samples were obtained between 8:00 and 9:00 a.m. after a 12-h period of overnight fasting. In control subjects, basal hormone levels were determined on cycle day 2–3. Hormone panels consisted of prolactin (PRL), thyroid-stimulating hormone (TSH), free thyroxine, LH, follicle-stimulating hormone (FSH), estradiol (E_2), P_4, 17α-hydroxyprogesterone (17α-OHP), testosterone, dehydroepiandrosterone sulfate (DHEAS), and sex-hormone binding globulin (SHBG). To assess glucose metabolism, glucose and insulin levels were measured while fasting and after an oral glucose tolerance test with a 75-g load of glucose.

4. Endometrial sampling

Endometrial samples were obtained with curettage or Pipelle endometrial aspirator (CooperSurgical, Trumbull, CT, USA) from the corpus of the uteri under sterile conditions. The control endometria were biopsied during the proliferative phase, on cycle day 10–12, due to morphological and physiological resemblances to the endometria of patients with PCOS. All endometrial specimens were diagnosed and dated by routine pathological analysis according to the Noyes criteria.[28] Tissue samples for RNA extraction were snap-frozen in liquid nitrogen and stored at -70℃.

5. Treatment of hESCs

The hESC line (CRL-4003) was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were plated in six-well cell culture plates at 2×10^5 cells per well in DMEM:F12 without phenol red, supplemented with 2% charcoal-stripped fetal bovine serum and 1% antibiotic-antimycotic solution. To mimic hyperandrogenism and hyperinsulinemia in patients with PCOS *in vitro*, hESCs were treated with 1, 10, or 100 μM DHT (Sigma-Aldrich, St. Louis, MO, USA) for 3 to 9 days, and 10 nM insulin (Sigma-Aldrich) for 8 hours, respectively. To evaluate the effects of long-term DHT, we chronically exposed hESCs to DHT for up to 4 weeks. Control cells received the vehicle, 0.1% dimethyl sulfoxide (DMSO).

6. *In vitro* decidualization of hESCs

Confluent hESC monolayers were treated with 0.5 mM N6, 2'-O-dibutyryladenosine cAMP (db-cAMP) and 1 μM medroxyprogesterone 17-acetate (MPA; both Sigma-Aldrich) for 1 to 9 days.

7. RNA extraction, RT-PCR and real-time RT-PCR

Total RNA was extracted from human endometrial tissues and hESCs using Trizol Reagent (Life Technologies, San Diego, CA, USA) according to the manufacturer's protocols. First-strand cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase and RNasin Ribonuclease Inhibitor (both Promega, Madison, WI, USA). Real-time PCR was performed to quantify expression levels, using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) on a Bio-Rad iCycler. To compare transcript levels between samples, a standard curve of cycle thresholds for several serial dilutions of a cDNA sample was established and used to calculate the relative abundance of each gene. In all experiments performed in this study, ribosomal protein L19 (rPL19) was used as a reference. All PCR reactions were performed in duplicate.

8. Western blot analyses

Cells were lysed in 150 μL lysis buffer including PRO-PREP Protein Extraction Solution (iNtRON, Seongnam, Korea) and 1× phosphatase inhibitor (Roche Applied Science, Indianapolis, IN, USA). Lysates were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10 μg/lane), transferred onto nitrocellulose membranes (Bio-Rad) and blocked with 5% non-fat milk (Bio-Rad) in TBS (Bio-Rad) containing 0.1% Tween 20 (Sigma-Aldrich). Membranes were incubated overnight at 4℃ with the following antibodies: GLUT1 (1:1000; Cell Signaling Technology, Denver, CO, USA); GLUT8 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA); GLUT12 (1:1000; Abcam, Cambridge, MA, USA); phospho-IRS1 (Tyr612; 1:1000; Santa Cruz Biotechnology); IRS1 (1:1000; Cell Signaling Technology); phospho-Akt (Ser473; 1:1000; Cell Signaling Technology); Akt (1:1000; Cell Signaling Technology); glyceradehyde-3-phosphate dehydrogenase (GAPDH; 1:2000; Cell Signaling Technology); β-Tubulin (1:2000; Cell Signaling Technology). Goat-anti rabbit and mouse IgG Horseradish Peroxidase (HRP)-conjugated secondary antibodies were diluted to 1:3000 in TBST with 5% milk and used for 1 h at room temperature (RT, 25℃). The signals were developed using the Clarity ECL Western Blotting Substrate Kit and detected using a ChemiDoc XRS+ system with Image Lab software (version 4.0) (all Bio-Rad).

9. Immunofluorescence analyses of hESCs

Cells were fixed in 4% paraformaldehyde and incubated with GLUT1 (1:100), GLUT8 (1:100), GLUT12 (1:50), and phospho-IRS1 (1:50) primary antibodies at 4℃ for 24 h. Cells were then washed in PBS and incubated with appropriate secondary antibodies conjugated to fluorescein isothiocyanate (FITC; 1:200; Jackson ImmunoResearch, West Grove, PA, USA). For negative controls, the primary antibodies were omitted, but incubation with secondary antibodies was retained. Nuclei were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI). Images were obtained using an Axio Imager 2 microscope with ZEN 2012 software (Carl Zeiss, Jena, Germany).

10. Microarray hybridization and data analyses for expression profiling with GSEA

Biotinylated cRNA were prepared from 500 ng total RNA according to the standard Affymetrix protocol (Expression Analysis Technical Manual, 2001, Affymetrix, Santa Clara, CA, USA). Following fragmentation, 12 μg of amplified RNA was hybridized for 16 h at 45°C on GeneChip PrimeView Human Gene Expression Arrays (Affymetrix), which contain over 530,000 probes representing approximately 20,000 well-characterized human genes, at the Biocore core facility (Seoul, Korea). GeneChips were washed and stained in the Affymetrix Fluidics Station 450, and scanned using the Affymetrix GeneChip Scanner 3000 7G. The data were analyzed with Robust Multi-array Analysis (RMA) using the default Affymetrix analysis settings and global scaling as the normalization method. The trimmed mean target intensity of each array was arbitrarily set to 100. The normalized and log transformed intensity values were then analyzed using GeneSpring GX 13.1 (Agilent Technologies, Santa Clara, CA, USA). Fold change filters included the requirement that the genes be present in at least 200% of controls for up-regulated genes and less than 50% of controls for down-regulated genes.

Expression values and detection calls were computed from the raw microarray data, and GSEA version 4.0 (Broad Institute, Cambridge, MA) was applied to interpret expression profiles.[29] GSEA was originally developed to identify cohorts of

genes whose functions are integrated into a certain biological process and/or signaling pathway. Pathways were ranked according to the significance of enrichment, and the validation mode measure of significance was used to identify pathways of greatest enrichment. Significance was tested by comparing the observed enrichment with that seen in data sets with randomly permutated sample labels (n=1000). Gene sets consisting of less than 15 or more than 500 genes were filtered out by gene set size filters.

11. Statistical analysis

Comparison groups were analyzed using unpaired Student's *t* and Mann-Whitney tests for parametric and non-parametric distributions, respectively. For multiple comparisons, ANOVA was used, followed by Tukey's honest significant difference or Games-Howell tests. In all cases, p-values<0.05 were considered significant.

III. RESULTS

1. Clinical characteristics and hormonal profiles

The clinical and hormonal characteristics of all subjects are presented in Table 1. Body mass index was similar between control subjects and patients with PCOS. Plasma levels of LH ($p<0.0001$) and testosterone ($p<0.01$) were significantly higher in patients with PCOS. Meanwhile, plasma levels of SHBG were lower in the PCOS group compared to the control group, but this was not statistically significant. Among glucose metabolism parameters, insulin levels 2 h after glucose load were significantly higher in patients with PCOS compared to control subjects ($p<0.05$).

Table 1. Comparison of the clinical characteristics and hormonal data in patients with PCOS and in normal cycling controls

Parameters	PCOS (N=16)	Control (N=7)	*P*-values
Age (yr)	28.1 ± 5.2	34.4 ± 2.0	0.008
BMI (kg/m^2)	24.8 ± 4.9	23.3 ± 4.1	0.624
E_2 (pg/ml)	54.1 ± 32.8	35.3 ± 13.4	0.089
FSH (mIU/ml)	6.55 ± 2.22	6.96 ± 1.13	0.720
LH (mIU/ml)	12.88 ± 6.00	3.96 ± 1.43	<0.0001
DHEAS (μg/dl)	211.0 ± 107.1	174.1 ± 75.4	0.452
Testosterone (ng/ml)	0.65 ± 0.27	0.29 ± 0.18	0.002
SHBG (nmol/liter)	39.0 ± 17.2	69.2 ± 39.8	0.089
Insulin 0 min (μU/ml)	8.56 ± 6.44	3.84 ± 2.95	0.055
Insulin 120 min (μU/ml)	64.4 ± 68.1	15.3 ± 6.9	0.040
Glucose 0 min (mg/dl)	99.5 ± 6.0	100.3 ± 5.3	0.579
Glucose 120 min (mg/dl)	121.4 ± 30.3	108.9 ± 17.0	0.278

Data expressed as the mean ± SD.

Table 2 shows a comparison of clinical and hormonal characteristics in lean and overweight/obese women with PCOS. As expected, the BMI of subjects in the overweight/obese PCOS group was significantly higher than in the lean PCOS group (p<0.0001). Plasma levels of LH, testosterone, and DHEAS were not statistically different between lean and overweight/obese PCOS patients. Meanwhile, plasma SHBG levels were significantly lower in the overweight/obese PCOS group compared with the lean PCOS group (p=0.001). Glucose and insulin levels 2 h after glucose load were higher in overweight/obese patients than lean patients (p=0.001).

Table 2. Comparison of clinical and hormonal characteristics in lean (lean PCOS) and overweight/obese (obese PCOS) women with PCOS

Parameters	Lean PCOS (N=9)	Obese PCOS (N=7)	*P*-values
Age (yr)	27.6 ± 4.9	28.7 ± 5.8	0.681
BMI (kg/m^2)	21.0 ± 1.9	29.6 ± 2.8	<0.0001
E_2 (pg/ml)	57.6 ± 39.2	49.6 ± 24.6	0.606
FSH (mIU/ml)	6.59 ± 2.46	6.50 ± 2.05	0.918
LH (mIU/ml)	14.8 ± 5.4	10.4 ± 6.1	0.114
DHEAS (μg/dl)	217.3 ± 99.7	202.8 ± 123.6	0.536
Testosterone (ng/ml)	0.73 ± 0.32	0.55 ± 0.17	0.174
SHBG (nmol/liter)	50.0 ± 14.3	25.0 ± 7.5	0.001
Insulin 0 min' (μU/ml)	4.25 ± 1.36	14.09 ± 6.14	0.003
Insulin 120 min (μU/ml)	21.3 ± 13.5	119.8 ± 70.5	0.001
Glucose 0 min (mg/dl)	100.4 ± 5.5	98.3 ± 6.8	0.299
Glucose 120 min (mg/dl)	102.4 ± 19.1	145.9 ± 23.8	0.001

Data expressed as the mean ± SD.

2. Up-regulation of IR and IRS expression in the endometria of patients with PCOS

To investigate the levels of proteins involved in the insulin signaling pathway, real-time RT-PCR was used to quantify IR, IRS1, and IRS2 mRNA levels in the endometria of controls and patients with PCOS. Primers used for RT-PCR and real-time RT-PCR are presented in Table 3. Expressions of IR ($p<0.05$), IRS1 ($p<0.01$), and IRS2 ($p<0.05$) mRNA were significantly higher in the endometria of patients with PCOS compared to control subjects (Fig. 1). Notably, there were no significant changes in IR, IRS1, or IRS2 mRNA expression between the endometria of lean and overweight/obese patients with PCOS (all $p>0.05$).

Table 3. Primers for RT-PCR and real-time RT-PCR

Gene name	Forward (5'→3')	Reverse (5'→3')	Amplicon (bp)
GLUT1	CCAGCTGCCATTGCCGTT	GACGTAGGGACCACACAGTTGC	98
GLUT3	CAATGCTCCTGAGAAGATCATAA	AAAGCGGTTGACGAAGAGT	172
GLUT4	CTGGGCCTCACAGTGCTAC	GTCAGGCGCTTCAGACTCTT	128
GLUT6	GCCCGGACTACGACACCT	AGCTGAAATTGCCGAGCAC	187
GLUT8	TCATGGCCTTTCTCGTGAC	TCCTTTAGTTTCAGGGACACAG	137
GLUT10	CTGTGGAGATACGAGGAAGA	TCAGTCCGTAGAGCAGGA	135
GLUT12	GGTACCTGTTGAAAACACCG	GCAGTGACAGATGACAGGAA	138
IR	GATGACAACGAGGAGTGTGG	GCAGCCGTGTGACTTACAGATG	156
IRS1	GCATCAGTTTCCAGAAGCAGC	CGTACCATCTACTGATGAGG	174
IRS2	CTTGTCCCACCACTTGAAGG	CACAGTCATTGCTCAGATCC	166
PRL	CGGAAGTACGTGGTATGCAAGA	TCAGGATGAACCTGGCTGACT	121
IGFBP1	CCAAACTGCAACAAGAATG	GTAGACGCACCAGCAGAG	87
rPL19	TGAGACCAATGAAATCGCCAATGC	ATGGACCGTCACAGGCTTGC	94

IGFBP, insulin-like growth factor-binding protein

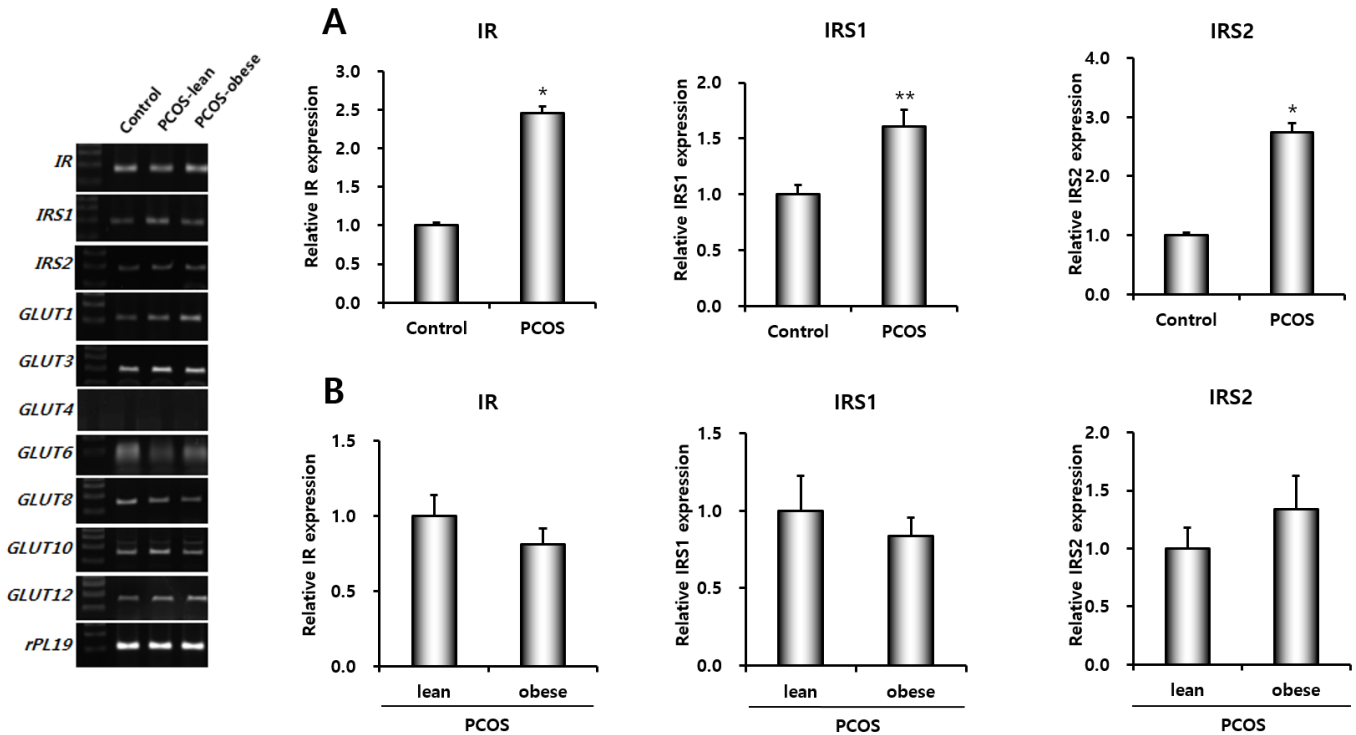


Figure 1. Semi-quantitative and real-time RT-PCR analyses of IR, IRS1, and IRS2 expression in the endometria of patients with PCOS (n=16) and in normally cycling controls (n=7). rPL19 was used as a reference. A. IR, IRS1, and IRS2 mRNA expression in the endometria of patients with PCOS compared to control subjects. B. IR, IRS1, and IRS2 mRNA expression in the endometria of lean and overweight/obese patients with PCOS. *, $p<0.05$ and **, $p<0.01$ compared to control subjects.

3. Up-regulation of GLUT1 and GLUT12 expression in the endometria of patients with PCOS

To determine the levels of GLUTs, including insulin dependent- and non-insulin-dependent types, mRNA levels for GLUT1, 3, 4, 6, 8, 10, and 12 were determined in the endometria of patients with PCOS and controls. GLUT1 ($p<0.05$) and GLUT12 ($p<0.001$) were significantly up-regulated in the endometria of PCOS patients compared to controls (Fig. 2). The expression of GLUT8 was also higher in patients with PCOS, but this was not statistically significant. GLUT4 mRNA was not detected in the endometria of patients with PCOS or control subjects; therefore, differences in GLUT4 expression levels could not be measured. There were no differences in GLUT expression between the endometria of lean and overweight/obese patients with PCOS.

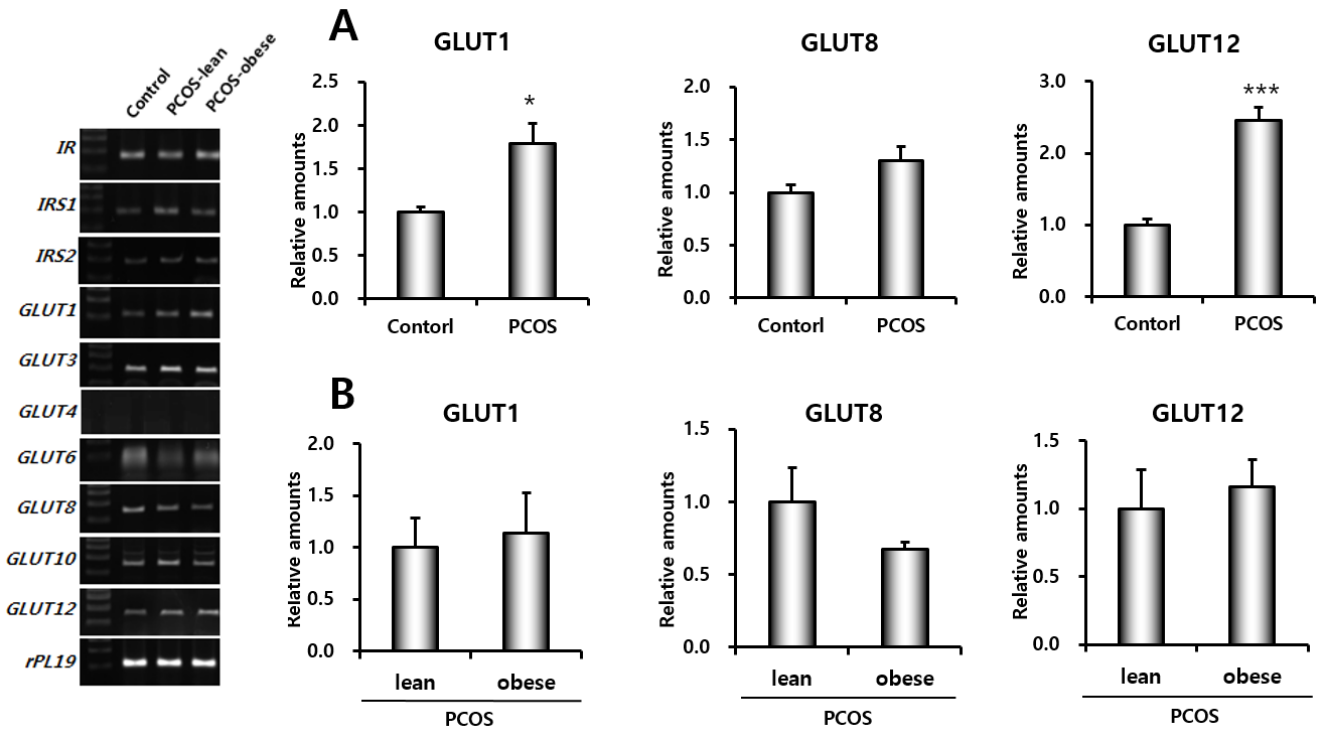


Figure 2. Semi-quantitative and real-time RT-PCR analyses of GLUTs expression in the endometria of patients with PCOS (n=16) and in normally cycling controls (n=7). GLUT1, 3, 4, 6, 8, 10, and 12 mRNA levels were determined. rPL19 was used as a reference. A. GLUT1, 8, and 12 expressions in the endometria of patients with PCOS and controls. B. GLUT1, 8, and 12 expressions in the endometria of lean and overweight/obese patients with PCOS. *, $p<0.05$ and ***, $p<0.001$ compared to control subjects.

4. Insulin and androgen treatment both up-regulate the expression of IRS1, Akt, and GLUTs in cultured hESCs

To determine the influences of hyperinsulinemia and hyperandrogenism on the human endometrium *in vitro*, I investigated protein levels of insulin signaling pathway factors and GLUTs in insulin- and androgen-treated hESC lysates. To do this, I employed immunofluorescence, real-time RT-PCR, and western blotting analyses in hESCs treated with 10 nM insulin for 8 hours to mimic hyperinsulinemia, or 1, 10, or 100 μM DHT for up to 9 days to mimic hyperandrogenism.

For immunofluorescence, paraformaldehyde-fixed hESCs were incubated with primary monoclonal antibodies against p-IRS1, GLUT1, GLUT8 and GLUT12 (Fig. 3). As a result, phosphorylation of IRS1 on Tyr^{612} increased after insulin- and androgen treatment in cultured hESCs, compared to vehicle controls. Immunofluorescence signal of GLUT1 increased in androgen-treated hESCs, but not in insulin-treated ones. Immunofluorescence signals of GLUT8 and GLUT12 in insulin- and androgen-treated hESCs were not significantly different from those corresponding to the control level.

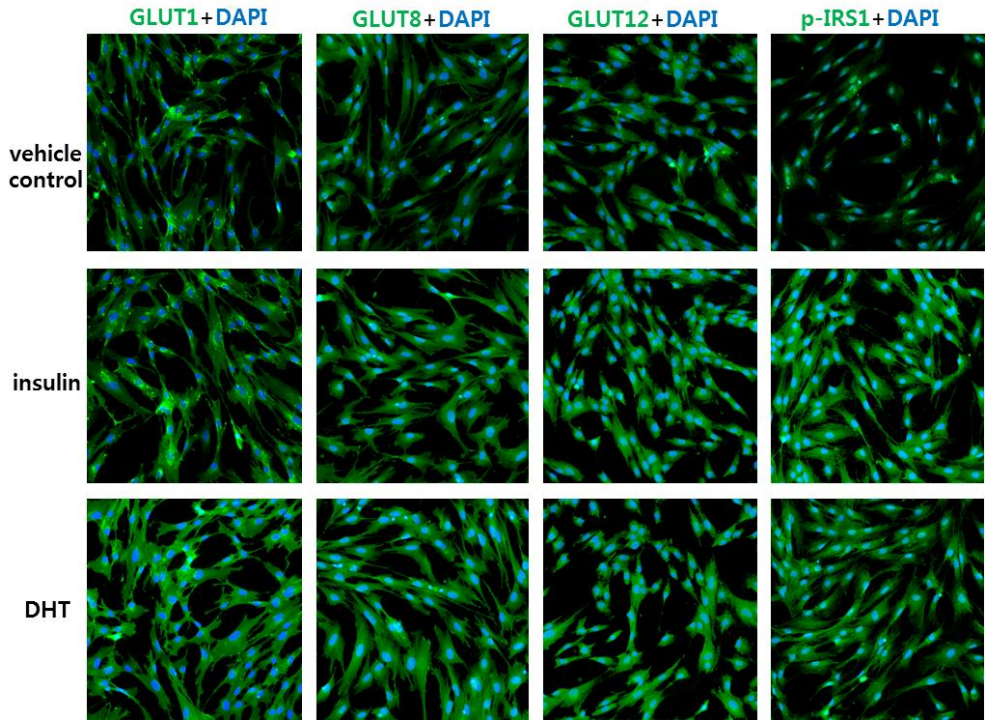


Figure 3. Immunofluorescence of p-IRS, GLUT1, 8, and 12 in cultured hESCs after treatment with 10 nM insulin for 8 hours or 1 μM DHT for 6 days. DAPI, 4',6-diamidino-2-phenylindole dihydrochloride.

To further examine the effects of insulin and androgen on the insulin-signaling pathway in human endometrium, western blot analyses were performed for cultured hESC lysates with antibodies against total and phosphorylated IRS1 and Akt (Fig. 4). Although total IRS1 mRNA expression after insulin or androgen treatment was similar to the control level, the p-IRS1 signal on Tyr^{612} increased in the insulin- and androgen-treated hESC lysates compared to the control. Similarly, the p-Akt signal on Ser^{473} was higher in the insulin- and androgen-treated hESC lysates. This suggests that insulin and androgen treatment increases the phosphorylation of IRS1 and Akt in hESCs.

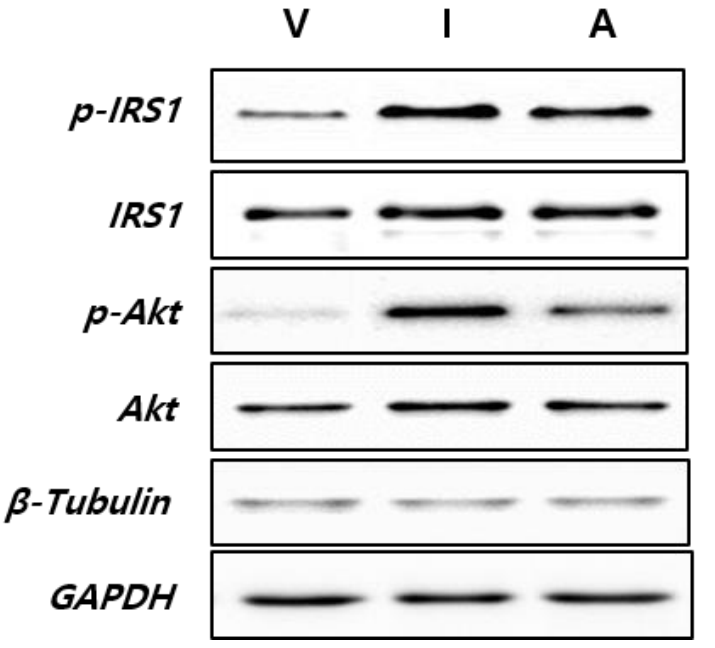


Figure 4. Western blot analyses of p-IRS1, total IRS1, p-Akt, and total Akt in cultured hESCs treated with 10 nM insulin for 8 hours or 1 μM DHT for 6 days. GAPDH and β-Tubulin were used as loading controls. V, vehicle control lysate; I, insulin-treated hESC lysate; A, DHT-treated hESC lysate.

5. Up-regulation of GLUT1 and GLUT12 in DHT-treated hESCs

To investigate the effects of androgens on the expression of GLUTs, I exposed hESCs to 1, 10, and 100 μM DHT for up to 9 days and determined the mRNA levels of GLUTs. The expression of GLUT1 was up-regulated in hESCs treated with 1 μM DHT on day 6 of treatment (Fig. 5); this effect had vanished by the 9th day of treatment. GLUT1 levels after treatment with 10 and 100 μM DHT was similar to the control level. The intensification effect of the androgen on GLUT12 expression was broader and more prolonged, as GLUT12 expression was up-regulated in 1 μM (on day 6 and 9) and 10 μM (on day 6 only) DHT-treated hESCs. The mRNA expression of GLUT8 was unchanged after DHT treatment.

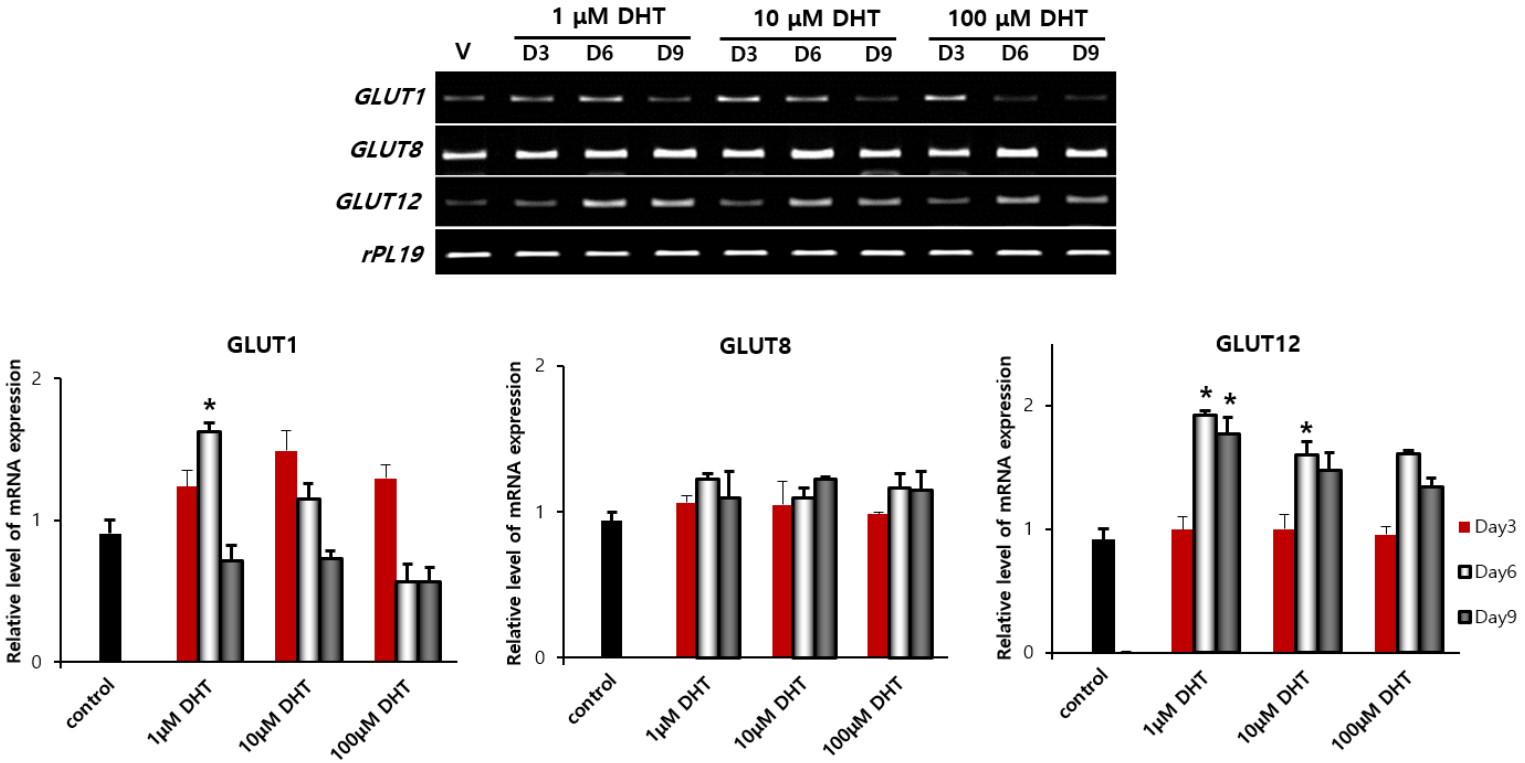


Figure 5. Semi-quantitative and real-time RT-PCR analyses of GLUTs in cultured hESCs after treatment with 1, 10, and 100 μM DHT for 3, 6, and 9 days. Control samples received 0.1% DMSO. rPL19 was used as a reference. *, $p<0.05$ compared to control samples.

6. Genome-wide expression profiles of androgen-treated hESCs

To examine how androgen affects the homeostasis of hESCs, the genome-wide expression profiles of hESCs cultured with (ESCs-T) or without (ESCs-C) DHT were compared. Unsupervised hierarchical clustering demonstrated that global expression patterns in ESCs-T and ESCs-C were distinctly different (Fig. 6A). Multiple probes for single genes were collapsed, resulting in a total of 11,027 genes for use in GSEA, which provided heat maps representing lists of the top 50 most increased and decreased genes in ESCs-T. Many genes involved in lipid metabolism and/or prostaglandin synthesis, such as *FABP4* (fatty acid binding protein 4), *ADH1B* (alcohol dehydrogenase 1B), *ATP8B4* (probable phospholipid-transporting ATPase IM), *PTGIS* (prostaglandin I_2 synthase) *PTGES* (prostaglandin E_2 synthase), and *PLA1A* (phospholipase A1) were included in the list of the 50 most increased genes in ESCs-T (Fig. 6B). Furthermore, genes induced by interferon(s) for immune responses, such as *IFIH1* (Interferon-induced helicase C domain-containing protein 1), *OAS1* (2'-5'-oligoadenylate synthetase 1), *OAS2*, *IFI27* (Interferon-alpha inducible protein 1), *IFI44L*, and *IFI6* were up-regulated by androgen treatment. Among the genes most decreased in ESC-Ts were a variety of cell cycle regulators, such as *IGF2* (insulin like growth factor 2), *CDC6* (cell division cycle 6), *CDK1* (cyclin dependent kinase 1), *MCM10* (mini-chromosome maintenance 10), *CDC45*, *E2F8* (E2F transcription factor 8), *E2F7*, *CDC25A*, and *CCNE2* (cyclin E2).

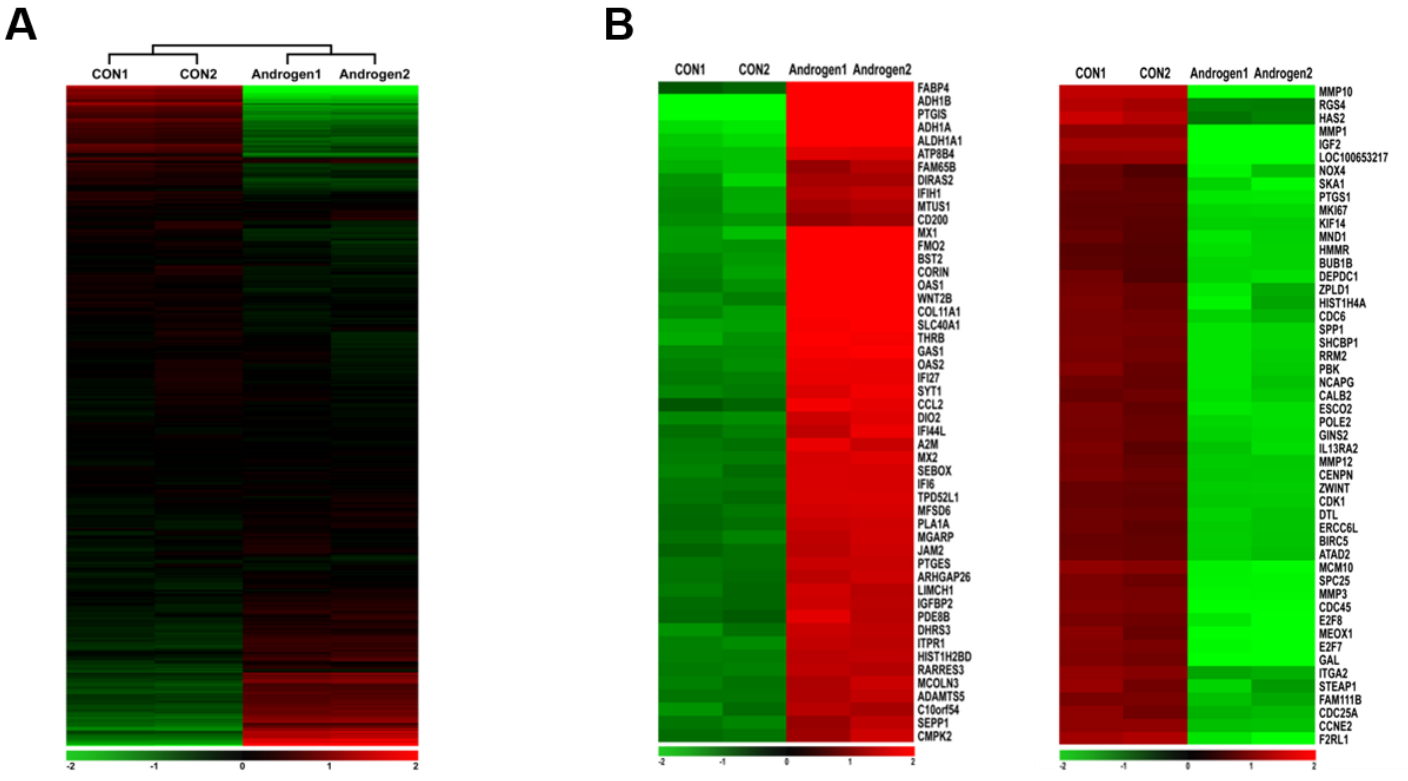


Figure 6. Global expression profiling in cultured hESCs after long-term treatment with DHT. A. Unsupervised hierarchical clustering analysis of mRNA microarray data from androgen-treated hESCs (ESCs-T) chronically exposed to DHT for up to 4 weeks. Control cells (ESCs-C) received 0.1% DMSO. B. Heat maps of the 50 most increased and decreased genes in ESCs-T. Green and red indicate low and high expression, respectively.

7. Identification of dysregulated signaling pathways in ESCs-T

To understand the underlying mechanisms by which androgen affects hESC homeostasis, it is critical to identify significantly dysregulated signaling pathways and biological processes. Supervised analyses, such as GSEA, provide such insights (Tables 4 and 5), as well as associated lists of differentially expressed genes. Table 4 shows a selective list from 604 gene sets enriched in ESCs-T, while Table 5 presents those enriched in ESCs-C from 687 gene sets, both with a false discovery rate of 25%. Both tables demonstrate that gene sets associated with glucose metabolism, cell cycle, estrogen receptor alpha targets, STAT3 targets, and inflammation were dysregulated by androgen treatment. For example, 'Glycolysis-gluconeogenesis', 'Transport of glucose and other sugars bile salts and organic acids metal ions and amine compounds', and 'IRS1 targets DN' gene sets were systemically up-regulated in ESCs-T, whereas 'Glucose transport', 'Regulation of glucokinase by glucokinase regulatory protein', and 'Glucagon signaling in metabolic regulation' were down-regulated by androgen treatment. I observed reduced expression of cell cycle regulators and increased expression of glycolysis-gluconeogenesis genes, depicted as heat maps in Figure 7. These results suggest that glucose transport and/or metabolism is dysregulated in hESCs by chronic androgen treatment, as is cell cycle regulation. Interestingly, gene sets known to be regulated by androgen signaling were not dysregulated in ESCs-T.

Table 4. A selective list from 604 gene sets enriched in ESCs-T

Name	Size	Nominal-*P*	FDR (%)
INTERFERON_SIGNALING	116	0	0
STEM_CELL_UP	184	0	0
STAT3_TARGETS_DN	39	0	0
ESR1_TARGETS_DN	446	0	0
ESTRADIOL_RESPONSE_24HR_DN	377	0	0.001
TEMPORAL_RESPONSE_TO_PROGESTERONE_CLUSTER_0	53	0	0.001
INFLAMMATORY_RESPONSE_AND_CHOLESTEROL_UP	29	0	0.002
REGULATED_BY_METHYLATION_UP	95	0	0.003
ENDMETRIUM_CANCER_DN	55	0	0.003
METASTASIS	26	0	0.004
VASCULAR_SMOOTH_MUSCLE_CONTRACTION	63	0	0.011
BREAST_CANCER_PROGRESSION_DN	56	0.002	0.013
DRUG_METABOLISM_CYTOCHROME_P450	20	0	0.015
CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	186	0	0.016
IRS1_TARGETS_DN	96	0	0.019
UTERINE_FIBROID_DN	48	0.004	0.019
OVARIAN_CANCER_POOR_SURVIVAL_UP	26	0.008	0.024
RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	44	0.002	0.039
HEDGEHOG_SIGNALING_PATHWAY	29	0.002	0.045
BIOLOGICAL_OXIDATIONS	51	0.002	0.047
LYSOSOME	101	0	0.048
MAMMARY_LUMINAL_MATURE_UP	60	0.008	0.065
GLYCOLYSIS_GLUCONEOGENESIS	39	0.006	0.129
CALCIUM_SIGNALING_PATHWAY	80	0.002	0.14
TRANSPORT_OF_GLUCOSE_AND_OTHER_SUGARS_BILE _SALTS_AND_ORGANIC_ACIDS_METAL_IONS _AND_AMINE_COMPOUNDS	31	0.008	0.15
FATTY_ACID_METABOLISM	29	0.033	0.21
TYROSINE_METABOLISM	19	0.049	0.224
CELL_ADHESION_MOLECULES_CAMS	52	0.031	0.229
TRYPTOPHAN_METABOLISM	21	0.041	0.235
ABC_TRANSPORTERS	19	0.073	0.241

FDR, false discovery rate

Table 5. A selective list from 687 gene sets enriched in ESCs-C

Name	Size	Nominal-*P*	FDR (%)
CELL_CYCLE	111	0	0
CHROMOSOME_MAINTENANCE	77	0	0
MRNA_PROCESSING	149	0	0
CERVICAL_CANCER_PROLIFERATION_CLUSTER	134	0	0
ESTRADIOL_RESPONSE_24HR_UP	269	0	0
BREAST_CANCER_GRADE_1_VS_3_UP	140	0	0
HYPOXIA_DN	263	0	0
ADIPOGENESIS_3	94	0	0
REGULATED_BY_METHYLATION_DN	112	0	0
EMBRYONIC_STEM_CELL_CORE	317	0	0
RESPONSE_TO_PROSTAGLANDIN_E2_UP	127	0	0
METASTASIS_UP	191	0	0
ESR1_TARGETS	66	0	0
ALL_GLUCOCORTICOID_THERAPY_DN	328	0	0
PYRIMIDINE_METABOLISM	84	0	0.001
GLUCOSE_TRANSPORT	31	0	0.001
REGULATION_OF_GLUCOKINASE_BY_GLUCOKINASE_REGULATORY_PROTEIN	24	0	0.001
TELOMERE_MAINTENANCE	41	0	0.001
ENDOTHELIUM_LYMPHATIC_VS_BLOOD_UP	93	0	0.001
TEMPORAL_RESPONSE_TO_PROGESTERONE_CLUSTER_14	130	0	0.004
PROGESTERONE_MEDIATED_OOCYTE_MATURATION	63	0	0.006
OOCYTE_MEIOSIS	87	0	0.006
ESR1_TARGETS_UP	19	0.01	0.02
CHOLESTEROL_BIOSYNTHESIS	19	0.016	0.021
EXTRACELLULAR_MATRIX_ORGANIZATION	48	0.006	0.022
MHC_CLASS_II_ANTIGEN_PRESENTATION	71	0.006	0.023
INFLAMMATORY_RESPONSE_LPS_DN	23	0.028	0.037
P53_SIGNALING_PATHWAY	59	0.004	0.054
GLUCAGON_SIGNALING_IN_METABOLIC_REGULATION	19	0.051	0.087
RNA_DEGRADATION	55	0.025	0.111
STAT3_TARGETS	22	0.091	0.151
POTASSIUM_CHANNELS	26	0.065	0.152
ENDOCRINE_THERAPY_RESISTANCE_4	206	0.022	0.171
PURINE_METABOLISM	106	0.028	0.19
INTRINSIC_PATHWAY_FOR_APOPTOSIS	27	0.092	0.203

FDR, false discovery rate

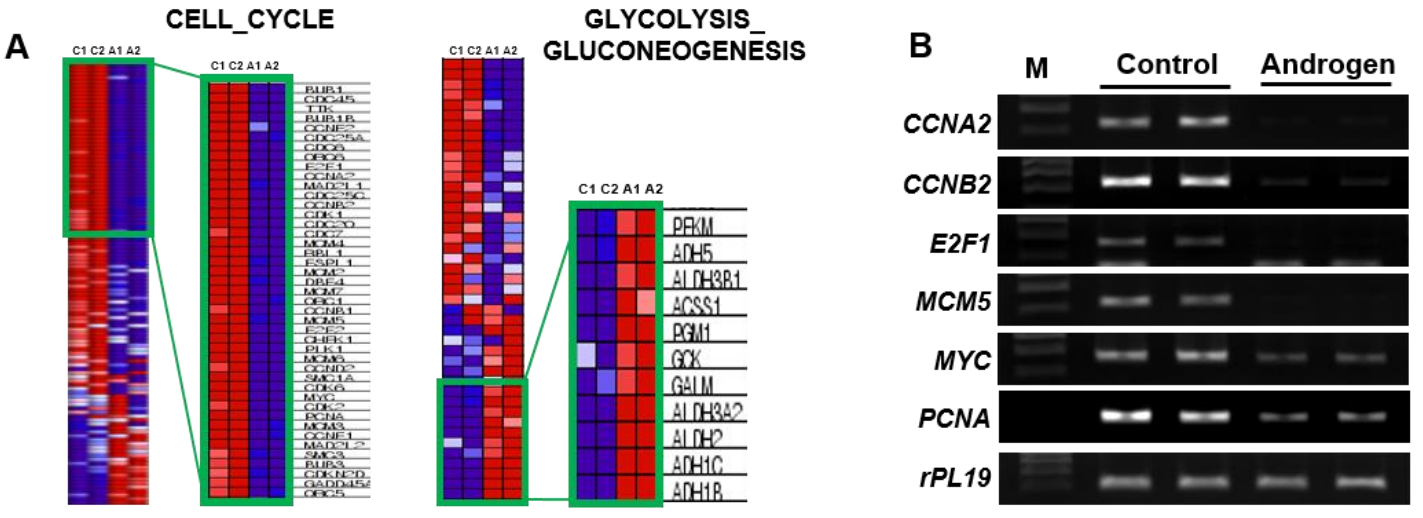
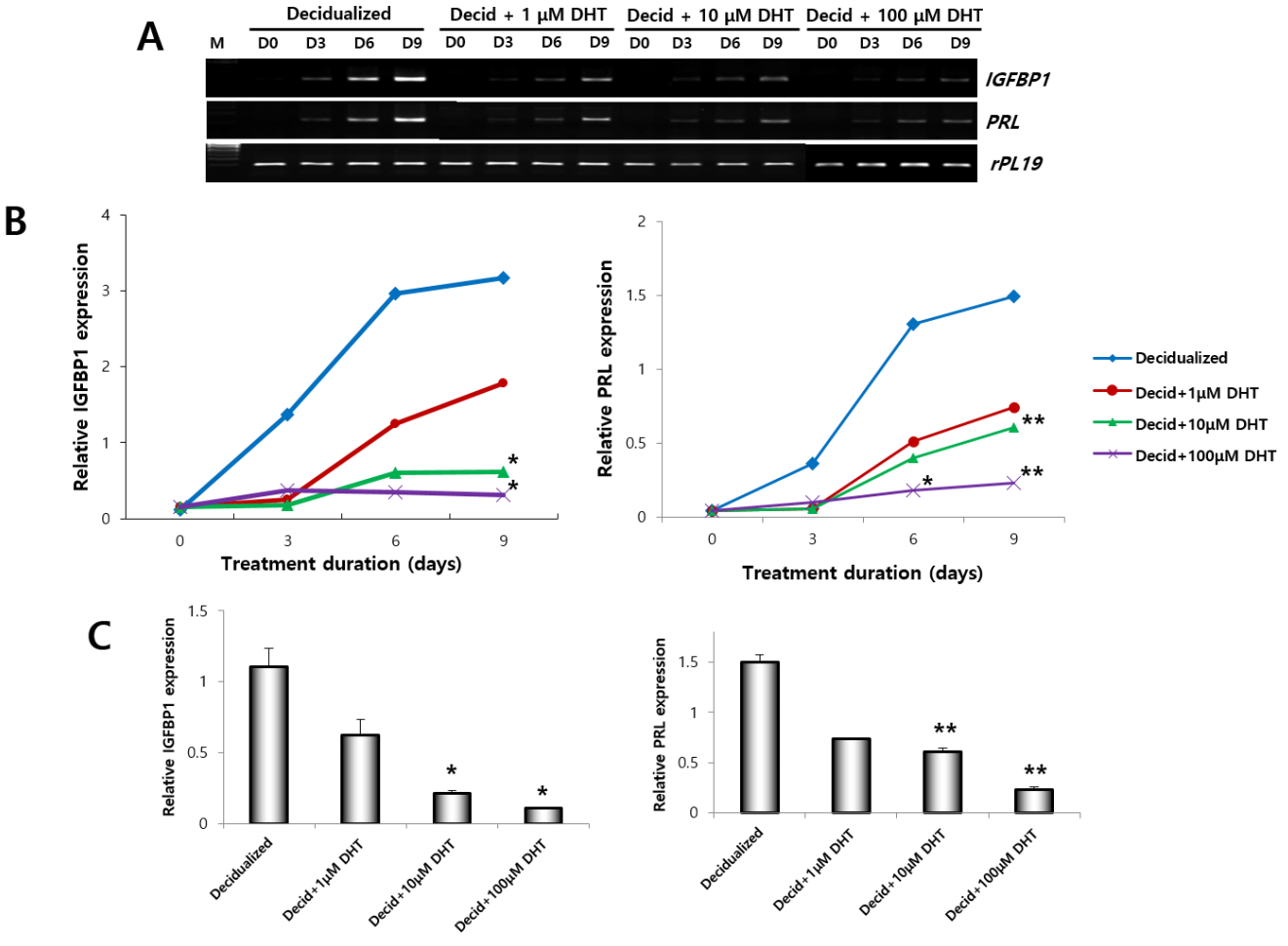


Figure 7. Effect of chronic androgen treatment on hESC gene expression. A. Heatmaps of cell cycle- and glycolysis/gluconeogenesis-related genes with expression changes after chronic exposure to DHT (A1, A2). Control samples (C1, C2) received 0.1% DMSO. Blue and red indicate low and high expression, respectively. B. Results of semi-quantitative RT-PCR for selected cell cycle-related genes. rPL19 was used as a reference.

8. DHT treatment inhibits decidualization of hESCs *in vitro*

As I observed no differences in insulin signaling between lean and overweight/obese patients with PCOS, I hypothesized that the hyperandrogenic PCOS environment may play a role in glucose metabolism and resultant decidualization, rather than insulin resistance or hyperinsulinemia. To investigate the effect of androgens on human endometrial decidualization, hESCs were cultured with 0.5 mM cAMP and 1 μM medroxyprogesterone-17-acetate for 3–9 days. At the time of *in vitro* decidualization, hESCs were co-treated with 1, 10, or 100 μM DHT (Fig. 8A and 8B). Notably, 10 and 100 μM DHT significantly decreased the mRNA expression of PRL and IGFBP1, well-known decidualization markers, on day 9 of culture (Fig. 8B and 8C). The inhibitory effects of androgen on hESC decidualization were also noted upon histologic observation of cultured hESCs (Fig. 8D). A morphological change was observed upon decidualization, as the cytoplasm increased and the cells became rounded, losing their fibroblastic phenotype. When exposed to 100 μM DHT, however, the decidualized hESCs lost the fibroblastic phenotype of decidualization, resembling the undecidualized control culture. Impaired *in vitro* decidualization by DHT was partially restored by flutamide, an androgen receptor antagonist (Fig. 9A and 9B). PRL and IGFBP1 levels, suppressed by long-term DHT treatment, were partially restored by co-treatment with flutamide.


A
Decidualized
Decid + 1 μM DHT
Decid + 10 μM DHT
Decid + 100 μM DHT
M
D0 D3 D6 D9
IGFBP1
PRL
rPL19
B
Relative IGFBP1 expression
Treatment duration (days)
Relative PRL expression
Treatment duration (days)
Decidualized
Decid+1μM DHT
Decid+10μM DHT
Decid+100μM DHT
C
Relative IGFBP1 expression
Relative PRL expression
Decidualized
Decid+1μM DHT
Decid+10μM DHT
Decid+100μM DHT

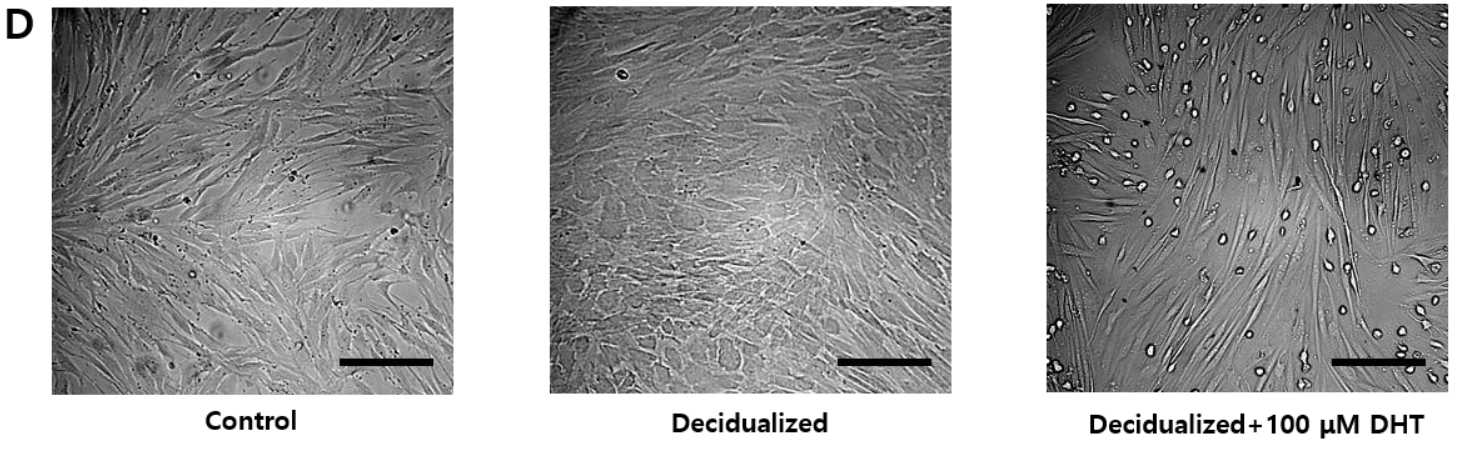


Figure 8. Effect of androgen treatment on decidualization of hESCs *in vitro*. To investigate the effect of androgens on decidualization of human endometrium, hESCs were cultured with 0.5 mM cAMP and 1 μM medroxyprogesterone-17-acetate for 3–9 days. At the time of *in vitro* decidualization, hESCs were co-treated with 100 μM DHT. Control samples received 0.1% DMSO. A and B. Real-time RT-PCR analysis of decidualization markers, IGFBP1 and prolactin (PRL), on day 3, 6, and 9 of DHT treatment. RPL19 was used as a reference. C. Decidualization marker expression on day 9. D. The decidual phenotype was assessed by phase-contrast microscopy. *, $p<0.05$ and **, $p<0.01$ compared to control samples. Scale bar: 200 μm.

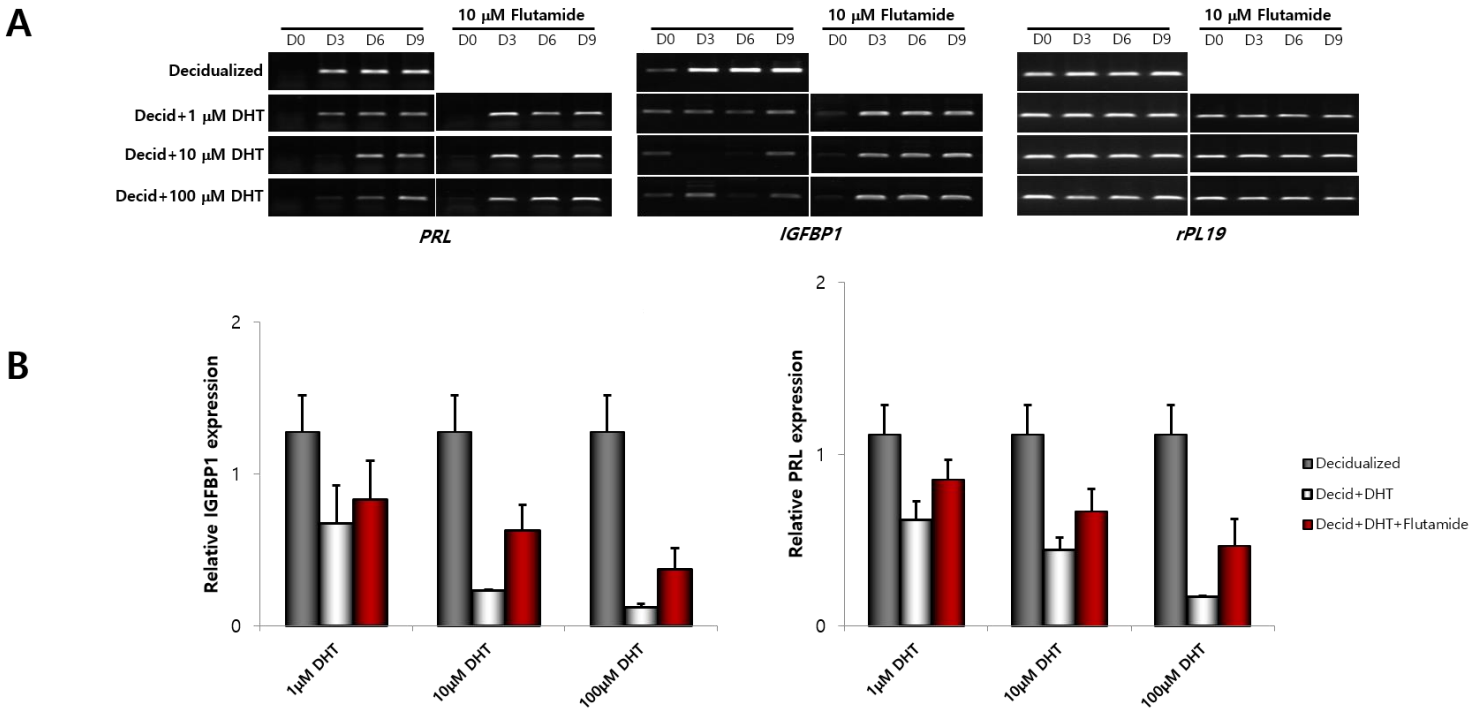
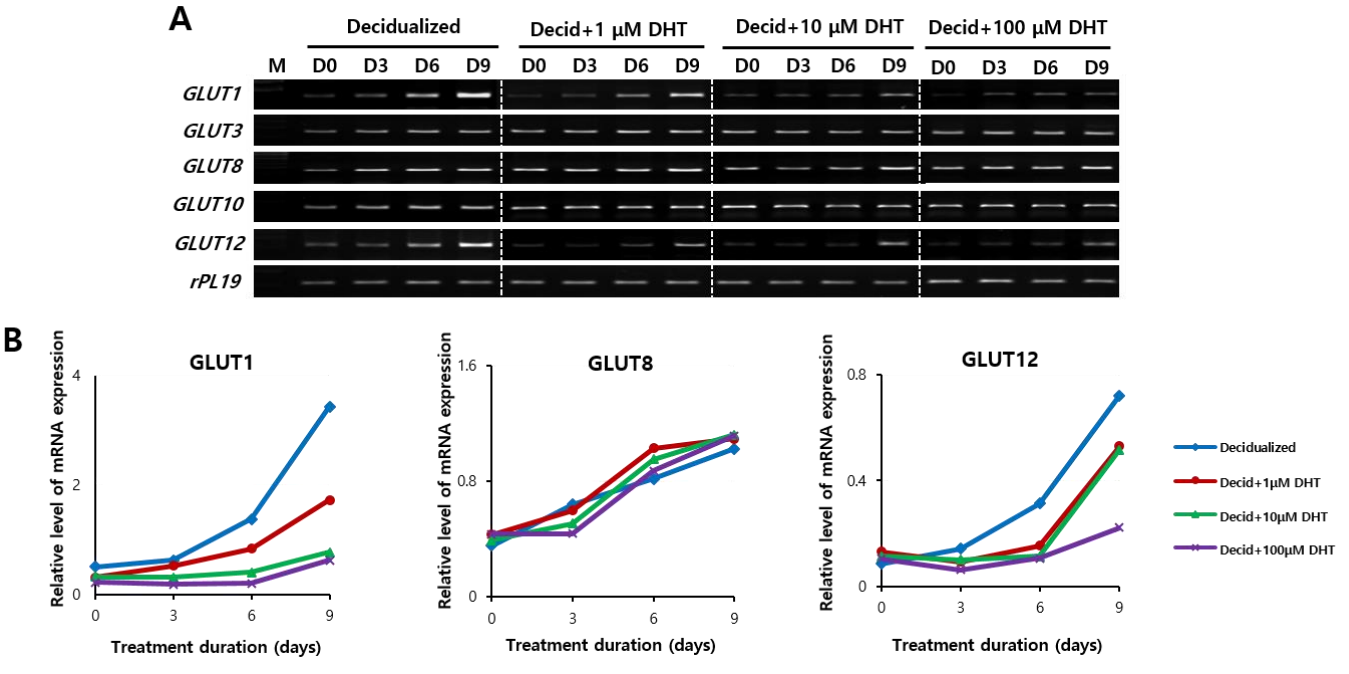


Figure 9. Effect of flutamide, an androgen receptor antagonist, on androgen-induced suppression of decidualization. To investigate the effect of androgens on decidualization of human endometrium, purchased hESCs were cultured with 0.5 mM cAMP and 1 μM medroxyprogesterone-17-acetate for 3–9 days. At the time of *in vitro* decidualization, hESCs were co-treated with 1, 10, or 100 μM DHT with or without 10 μM flutamide. A and B. Semi-quantitative and real-time RT-PCR analyses of decidualization markers. Control samples received 0.1% DMSO. rPL19 was used as a reference.

9. GLUT1, 8, and 12 are up-regulated in decidualizing hESCs and DHT treatment suppresses the expression of GLUT1 and 12

Among five GLUTs analyzed, the expression levels of GLUT1, 8, and 12 were gradually increased during *in vitro* decidualization (Fig. 10A). In contrast with undecidualized endometria from patients with PCOS, GLUT1 and 12 protein and mRNA levels were decreased when decidualizing hESCs treated with DHT (Fig. 10B and 10C); however, these changes were not statistically significant.



C

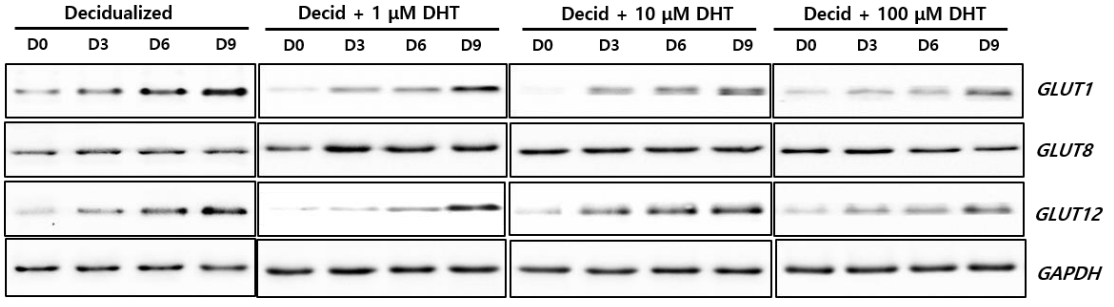


Figure 10. Effect of androgen treatment on the expression of GLUTs during *in vitro* decidualization of hESCs. To investigate the effect of androgens on decidualization of human endometrium, purchased hESCs were cultured with 0.5 mM cAMP and 1 μM medroxyprogesterone-17-acetate for 3–9 days. At the time of *in vitro* decidualization, hESCs were co-treated with 1, 10, or 100 μM DHT. Control samples received 0.1% DMSO. A and B. Semi-quantitative and real-time RT-PCR analysis of GLUT mRNA levels on day 3, 6 and 9 of DHT treatment. rPL19 was used as a reference. C. Cell lysates were analyzed by western blotting using antibodies against GLUT1, 8, and 12. GAPDH was used as a loading control.

IV. DISCUSSION

Many studies have suggested that proper glucose metabolism is an important factor in endometrial differentiation and successful blastocyst implantation.[16,17,25] Cellular uptake by facilitative glucose transporters is a critical initial step in glucose utilization. The 14 GLUT proteins, which are members of the solute carrier 2 (SLC2) family, are characterized by the presence of 12 membrane-spanning helices and several conserved sequence motifs.[16,30] In this study, we investigated seven different family members, GLUT1, 3, 4, 6, 8, 10, and 12, which have been previously identified in human endometrium.[16] To the best of our knowledge, this is the first work presenting a detailed and comprehensive quantification of a series of GLUTs in the endometria of patients with PCOS.

The underlying molecular mechanism behind the increase in GLUT1 in the endometria of patients with PCOS is not known. GLUT1 is responsible for basal glucose uptake and nutritional support in all cell types, independent of the insulin signaling pathway.[31] Since its first detection in human endometrium, increased endometrial GLUT1 expression has typically been reported in conditions with high energy demand, such as decidualization, and endometrial adenocarcinoma.[17,25,26,32-35] Considering this, we suggest that the increased energy demand associated with chronic stimuli from hyperestrogenic and hyperinsulinemic conditions in patients with PCOS might contribute to increased GLUT1 expression in the endometrium.

Notably, GLUT4 mRNA was not detected in the endometria of either PCOS patients or control subjects. Furthermore, we observed increased expression of IR and IRS1 mRNA in the endometria of patients with PCOS. GLUT4 is the predominant GLUT in insulin-sensitive tissues such as striated muscle and adipose tissue. Many studies have reported reduced levels of GLUT4 and IRS1 mRNA in the endometria of patients with PCOS.[19-24,36,37] Decreased IRS1 protein expression upon testosterone treatment was reported in *ex vivo* cultured endometrial epithelial cells.[37] Based on these findings, the authors suggested that the uterine endometrium is also an insulin-sensitive tissue. However, the findings in my experiments are inconsistent with these results, but consistent with other studies reporting undetectable levels of GLUT4 in human endometria and endometrial stromal cells.[17,20,25] Mozzanega et al (2004) reported a decreased but detectable level of GLUT4 expression in epithelial cells from the endometria of patients with PCOS, but failed to detect GLUT4 expression in the stromal cells.[20] Given the imperative role of stromal cells in the process of decidualization, GLUT4 might be less important for uterine receptivity than GLUT1 and 12.

As mentioned above, I observed increased expression of IR and IRS1 mRNA in the endometria of patients with PCOS. This is in contrast with reports of reduced IRS1 mRNA in the endometria of patients with PCOS.[36,37] However, *in vitro* analysis using human endometrial stromal cell lines in my experiments supports the results obtained on analyzing the endometria of patients with PCOS. I investigated the levels of

GLUTs and factors involved in the insulin signaling pathway by immunofluorescence, real-time RT-PCR, and western blotting analyses in hESCs treated with insulin or androgen, to mimic hyperinsulinemia or hyperandrogenism, respectively. These experiments demonstrate that the signals of p-IRS1 on Tyr^{612} and p-Akt on Ser^{473} increased in the insulin- and androgen-treated hESC lysates, compared to the control. This suggests that hyperinsulinemic as well as hyperandrogenic conditions increases the phosphorylation of IRS1 and Akt in human endometrium. In addition, I also demonstrate that the mRNA expression of GLUT1 and GLUT12 was up-regulated in androgen-treated hESC lysates. Interestingly, Corbould et al. reported the increased IRS1 protein abundance in the skeletal muscle of women with PCOS, which results in decreased phosphatidylinositol 3-kinase (PI3K) activity.[38] In that study, phosphorylation of IRS1 on Ser^{312} was significantly increased in PCOS, which contributed to insulin-signaling and insulin-stimulated glucose uptake defects. Although my experiments show a similar increase in IRS1 mRNA expression in androgen-treated hESCs, the detailed phosphorylation patterns are different from those reported by Corbould et al.[38] It is well-known that Tyr^{612} is important for IRS1 to activate the PI3K-dependent pathway, and finally mediate the translocation of GLUT4 in response to insulin.[39] Tyrosine phosphorylation of IRS1 plays a positive role in insulin signaling. In my experiments, I observed an increase in the levels of Tyr^{612}-phosphorylated p-IRS1 and Ser^{473}-phosphorylated p-Akt in androgen-treated hESC lysates, which is not consistent with the theory that the uterine endometrium is an insulin-sensitive tissue. However, further studies are needed to identify a more specific

mechanism that can corroborate these findings.

I also found an increased level of GLUT12 mRNA in the endometria of patients with PCOS. GLUT12 is a recently discovered member of the SLC2 family, and has been demonstrated to be insulin-sensitive in human muscle cells and transgenic mice.[40,41] Considering that insulin resistance is an innate feature of PCOS, the underlying mechanism behind increased GLUT12 expression in the endometria of patients with PCOS remains unknown. Interestingly, one study demonstrated that insulin stimulation increased translocation of GLUT4 but not GLUT12 in healthy myometrial cells;[42] in addition, they observed an increased level of cell surface GLUT12 in diabetic myometrium, potentially as a compensatory mechanism for the observed down-regulation of GLUT4. Like this study, my results also suggest that in contrast to GLUT4, GLUT12 functions as an insulin-independent glucose transporter in human endometrial tissue.

Similar to the results of several previous studies, my experiments demonstrate increased expression of GLUT1 during *in vitro* decidualization.[17,25,26] Notably, I originally demonstrated increased expression of GLUT12 mRNA during *in vitro* decidualization process. These results further support the hypothesis that, similar to GLUT1, GLUT12 functions as an insulin-independent glucose transporter in human endometrium. Remarkably, DHT treatment in my study inhibited increased expression of GLUT1 and GLUT12 in decidualizing hESCs. Although my *in vitro* study suggests

that a hyperandrogenic endometrial environment suppresses both decidualization and glucose metabolism, no assumption of causal inference between the two can be drawn from these results. Nevertheless, it could be suggested that a hyperandrogenic endometrial environment might adversely affect decidualization, and consequently glucose utilization, in patients with PCOS.

I investigated the genome-wide expression profiles of endometrial stromal cells cultured with androgen (ESCs-T) or a vehicle control (ESCs-C). According to selective lists from gene sets that were enriched in ESCs-T and ESCs-C, gene sets associated with glucose metabolism, cell cycle, estrogen receptor alpha targets, STAT3 targets, and inflammation were dysregulated by androgen treatments (Tables 4 and 5); some of these results are similar to those in a previous study of PCOS-affected endometria.[27] Semi-quantitative RT-PCR analysis in my experiments demonstrated that proteins imperative for cell cycle progression, including cyclins (CCNA2 and CCNB2), minichromosome maintenance proteins (MCM2), and the transcription factor E2F1 are collectively down-regulated in ESCs-T (Fig. 7B). These results suggest that decreased cell cycle regulators in the endometria of patients with PCOS might be related to hyperandrogenism rather than hyperinsulinemia. Although these data demonstrate the influence of hyperandrogenic condition on insulin-signaling pathway and glucose transporters in the human endometrium, the underlying mechanisms by which a hyperandrogenic environment influences glucose metabolism in non-pregnant versus decidualized endometrium remain incompletely defined.

V. CONCLUSON

Increased abundance of IR, IRS, GLUT1, and GLUT12 proteins in the endometria of patients with PCOS suggest the influence of hyperandrogenic milieu on insulin signaling pathway and glucose utilization. Also, *in vitro* analysis using human endometrial stromal cells supports the results obtained on analyzing the endometria of patients with PCOS. Of note, mRNA microarray experiments analyzed using GSEA reveal androgen-induced dysregulation of glucose transport and/or metabolism in human endometrium. In decidualization, hyperandrogenic conditions down-regulated the expression profiles of GLUT1 and 12, possibly impairing decidualization and decreasing uterine receptivity.

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ABSTRACT(IN KOREAN)

다낭성난소증후군 환자 자궁내막세포의 인슐린 신호전달계 및 당전달체 조절에 관한 연구

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연구목적

다낭성난소증후군 환자의 가임력 감소는 난소의 배란기능 이상 외에도 배아착상 및 임신유지에 영향을 주는 자궁내막의 수용능력장애와도 연관될 것으로 추정되고 있다. 이전의 일부 연구에서 다낭성난소증후군 환자의 인슐린저항성이나 남성호르몬과다가 임신초기 착상실패와 관련된다고 보고된 바 있으나 아직까지 다낭성난소증후군 환자 자궁내막의 항상성 및 병태생리에 대해 포괄적인 규명이 이루어지지 못한 실정이다. 본 연구에서는 다낭성난소증후군 환자의 자궁내막에서 인슐린민감성 및 당전달체 (GLUT) 발현의 특성을 확인하고 다낭성난소증후군의 생리 특성인 고안드로겐환경이 자궁내막기질세포의 당 대사 및 탈락막화 과정에 미치는 영향을 규명하고자 하였다.

연구설계

본 연구는 인간에서 채취한 자궁내막 조직과 인간자궁내막기질세포주를 대상으로 하였다.

연구방법

인간 자궁내막 조직은 7명의 정상군과 16명의 다낭성난소증후군 환자에서 무균적으로 채취하였으며 인슐린 신호전달 및 당 대사 과정에 관여하는 주요인자들의 발현 및 활성을 중합효소연쇄반응과 단백질흡입법을 이용해 조사하였다. 생체 외 고안드로겐 및 고인슐린환경은 인간자궁내막기질세포주에 디하이드로테스토스테론과 인슐린을 처리하여 조성하였으며 탈락막화 과정은 초산메드록시프로게스테론으로 유도하였다. 배양된 자궁내막기질세포의 유전자발현 변화는 알엔에이 마이크로어레이를 통한 유전자집합농축분석법을 이용해 조사하였다.

연구결과

다낭성난소증후군 환자의 자궁내막에서 정상군에 비해 GLUT1, GLUT12, 인슐린수용체 및 인슐린수용체기질의 발현이 유의하게 증가하였으며 다낭성난소증후군 환자 중 비만군과 정상체중군 간의 비교에서는 이들 단백의 발현에 차이를 보이지 않았다. 인슐린민감성의 지표가 되는 GLUT4는 정상군과 다낭성난소증후군 환자군 자궁내막

조직에서 모두 발현되지 않았다. 생체 외 인간자궁내막기질세포주에 고안드로겐 및 고인슐린 환경을 각각 조성하는 경우 인슐린수용체기질1과 Akt의 발현이 증가하였고 GLUT1과 GLUT12의 발현은 고안드로겐 환경하에서 증가하였다. 생체 외 인간자궁내막기질세포주에 탈락막화 과정을 유도하는 경우 GLUT1, GLUT8 및 GLUT 12의 발현 증가가 확인되었으며 탈락막화 과정에 고안드로겐환경을 조성하는 경우 탈락막화 과정뿐만 아니라 GLUT1과 GLUT12의 발현도 억제되었다. 또한 고안드로겐환경에 의한 당 대사 관련 유전자의 조절장애와 세포주기조절인자 관련 유전자의 감소가 알엔에이 마이크로어레이를 통해 확인되었다.

결론

다낭성난소증후군 환자의 자궁내막에서 정상인에 비해 인슐린신호연결단백과 당전달체의 발현이 비정상적으로 증가하는 결과가 확인되었으며 특히 여러 당전달체의 발현 특성이 처음으로 규명되었다. 또한, 본 연구에서 이용한 알엔에이 마이크로어레이를 통해 자궁내막기질세포에서 안드로겐에 의한 일부 당 전달 및 대사 관련 유전자의 조절장애가 확인되었다. 인간자궁내막세포주의 탈락막화 유도 중 고안드로겐 환경을 조성하는 경우 탈락막화 과정이 억제되어 자궁내막의 수용능력 장애가 초래되었는데 이 과정에서 당전달체1과 12의 발현의 억제가 확인되어 다낭성난소증후군 환자의 고안드로겐환경에

의한 임신 착상의 장애 발생 기전의 중요 중간 연결과정이 확인되었다.

핵심 되는 말: 자궁내막, 다낭성난소증후군, 당전달체, 안드로겐, 탈락막화