Differential expression of protein disulfide isomerase (PDI) in regulating endometrial receptivity in humans

Sudini R Fernando[a,b], Kottawattage SA Kottawatta[a,c], Luhan Jiang[a], Xian Chen[a], Kiu-Wai Cheng[a], Benancy PC Wong[a], Ernest HY Ng[a,d], William SB Yeung[a,d], Kai-Fai Lee [a,d,*]

[a] Department of Obstetrics and Gynaecology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China

[b] Department of Animal Science, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka

[c] Department of Veterinary Public Health and Pharmacology, Faculty of Veterinary Medicine and Animal Science, The University of Peradeniya, Peradeniya, Sri Lanka

[d] Shenzhen Key Laboratory of Fertility Regulation, The University of Hong Kong-Shenzhen Hospital, Futian District, Shenzhen, China

* Correspondence: Kai-Fai Lee, Department of Obstetrics and Gynaecology, The University of Hong Kong, L7-49, Laboratory Block, FOMB, 21 Sassoon Road, Pokfulam, Hong Kong, China. Fax: 852-28161947 and E-mail: ckflee@hku.hk

Abstract

Estrogen and progesterone regulate the expression of endometrial proteins that determine endometrial receptivity for embryo implantation. The protein disulfide isomerase (PDI) family of proteins play a diverse role in regulating protein modification and redox function. Although the role of PDIs in cancer progression has been widely studied, their role in endometrial receptivity is largely unknown. We have focused on the expressions of PDIA1, PDIA2, PDIA3, PDIA4, PDIA5, and PDIA6 isoforms in endometrial epithelium under the influence of estrogen and progesterone and investigated their functional role in regulating endometrial receptivity. We found PDIA1-6 transcripts were expressed in endometrial epithelial Ishikawa, RL95-2, AN3CA, and HEC1-B cell lines. The expression of PDIA1 was low and PDIA5 was high in HEC1-B cells, whereas PDIA2 was high in both AN3CA and HEC1-B cells. In Ishikawa cells, estrogen (10 and 100 nM) upregulated PDIA1 and PDIA6, whereas estrogen (100 nM) downregulated PDIA4 and PDIA5; and progesterone (0.1 and 1 μM) downregulated transcript expressions of PDIA1-6. In human endometrial samples, significantly lowered transcript expressions of PDIA2 and PDIA5 were observed in the secretory phase compared with the proliferative phase, whereas no change was observed in the other studied transcripts throughout the cycle. Inhibition of PDI by PDI antibody (5 and 10 μg/mL) and PDI inhibitor bacitracin (1 and 5 mM) significantly increased the attachment of Jeg-3 spheroids onto AN3CA cells. Taken together, our study suggests a role of PDI in regulating endometrial receptivity and the possibility of using PDI inhibitors to enhance endometrial receptivity.

Key words: PDI, Estrogen, Progesterone, PDI inhibitors, Endometrial receptivity

1. Introduction

Endometrial receptivity is a tightly regulated phenomenon that plays a vital role in embryo implantation and pregnancy success [1]. Predicting endometrial receptivity before embryo transfer is one of the challenging research questions that remains unanswered. Different transcriptomic [2], proteomic [3, 4], microbiomic [5, 6], and lipidomic [7, 8] approaches have been used to ascertain the endometrial receptivity in women undergoing fertility treatment. However, the underlying molecular mechanism leading to a receptive endometrium remains largely unknown.

Estrogen and progesterone play major roles in regulating endometrial receptivity [9, 10], with the proliferative phase of the menstrual cycle dominated by estrogen regulation and the secretory phase by progesterone regulation. Estrogen and progesterone also regulate the expression of genes through classical and non-classical steroid receptor signaling pathways [10]. Interestingly, the classical ligand-receptor binding pathways also play vital roles in regulating endometrial receptivity [11].

Different endometrial biomarkers have been identified to predict the endometrial receptivity. Transcriptional factors such as HOXA genes are promising markers in predicting endometrial receptivity [12], with HOXA-10 and HOXA-11 playing a major role in implantation [13]. Higher expression of HOXA-10 and HOXA-11 is observed in the mid-luteal phase of the human menstrual cycle [13, 14], and significantly lower HOXA-10 and HOXA-11 transcripts expression has been observed in infertile patients [15]. Moreover, lower HOXA-10 and HOXA-11 transcripts expression is observed in infertile women with endometrioma [16] and lower HOXA-10 transcripts expression is observed in patients with endometriosis [17].

Protein disulfide isomerases (PDIs) are a family of proteins with 21 isoforms with multiple functions including in redox reactions, chaperone activity, and protein folding [18]. They are mainly located in the endoplasmic reticulum of cells and are also present in the nucleus, cytosol, and cell membrane [19]. Many studies have reported that PDIs have a role in cancer cell progression [20]. Moreover, several PDI isoforms including PDIA1, PDIA3, or PDIA6 are regulated by estrogen in cancer cells [21]. It has been reported that PDIA3 is expressed in human endometrial epithelial cell lines RL95-2 and HEC1-B [22].

Due to the limited availability of human endometrial epithelial cells and embryos for research, studies have widely used endometrial and trophoblastic cell lines. The phenotype and molecular properties of endometrial epithelial cells are different during the receptive and non-receptive phases. Thus, different cell lines have been developed for this purpose. Ishikawa, RL95-2, HEC1-B, and AN3CA are the most used endometrial epithelial cell lines. Ishikawa and RL95-2 are endometrial epithelial cell lines that exhibit properties of a receptive endometrium, whereas HEC1-B and AN3CA cell lines exhibit characteristics of a non-receptive endometrium [23]. These cell lines differ from each other in terms of their adhesiveness toward trophoblastic spheroids such as Jeg-3 or JAr spheroids, with Ishikawa and RL95-2 exhibiting a higher spheroid attachment rate [24], while HEC1-B [25] and AN3CA [26] have a lower spheroid attachment rate. In addition, these receptive and non-receptive cell lines differ in their expression of adhesive and anti-adhesive molecules and steroid receptors [23]. There have been no reports to date of the expression and steroid regulation of PDI isoforms in human endometrial cell lines and endometrial tissues. Therefore, this study focused on the analysis of the differential expression of transcripts of PDIA1, PDIA2, PDIA3, PDIA4, PDIA5, and PDIA6 in endometrial epithelial cells to delineate their functional role in regulating endometrial receptivity.

2. Materials and Methods

2.1. Human endometrial epithelial cell lines

Human endometrial epithelial Ishikawa (ECACC 99040201), RL95-2 (CRL1671, ATCC), AN3CA (HTB-111, ATCC), and HEC1-B (HTB-113, ATCC) cells, and human trophoblastic Jeg-3 cells (HTB-36, ATCC) were used in this study. Ishikawa, AN3CA, and HEC1-B cells were maintained in Minimum Essential Medium (MEM, M0268, Sigma) supplemented with 1% Penicillin/Streptomycin (15140-122, Thermo Fisher), 1% L-glutamine (25030-081, Thermo Fisher), and 10% fetal bovine serum (FBS, 10270, Thermo Fisher). Both RL95-2 and Jeg-3 cells were maintained in Dulbecco's Modified Eagle Medium nutrient mixture F12 (DMEM F12, D8900, Sigma) supplemented with 1% Penicillin/Streptomycin (15140-122, Thermo Fisher), 1% L-glutamine (25030-081, Thermo Fisher), and 10% FBS (10270, Thermo Fisher). All cells were sub-cultured every 2-3 days and maintained at 37°C in 5% CO_2.

2.2. Total, membrane, and cytoplasmic protein extraction from endometrial cells

Total cell protein from Ishikawa, RL 95-2, AN3CA, and HEC1-B was extracted using Radio Immunoprecipitation Assay buffer solution (1× phosphate-buffered saline (PBS), 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease inhibitors. Membrane proteins were extracted using the Native Membrane Protein Extraction Kit (ProteoExtract-Native Membrane Protein Extraction Kit, 444810, Sigma). Briefly, $3x10^5$ cells were collected and washed two times with 1X Tris-buffered saline with centrifugation at 600 g for 10 minutes for each wash. Cytosolic proteins were separated by adding 2 mL of lysis buffer I with 10 µL protease inhibitor cocktail to the cells followed by incubation for 15 minutes and

centrifugation at 16000 g for 30 minutes. The supernatant was collected. Next, 1 mL of lysis buffer II with 5 µL protease inhibitor cocktail was added to the remaining pellet and incubated for 30 minutes with intermittent shaking followed by centrifugation at 16000 g for 30 minutes. The supernatant enriched with membrane proteins was collected. Protein concentrations were measured using Pierce BCA assay (Pierce BCA protein assay kit, 23225, Thermo Fisher) before Western blot analysis.

2.3. SDS-Polyacrylamide gel electrophoresis and Western blotting

Total/membrane/cytosolic proteins were denatured by adding 5X SDS loading buffer and heating at 95°C for 10 minutes. The denatured proteins were separated by 8% SDS PAGE at a constant 60 mA. The separated proteins in the gel were transferred to a PVDF membrane for 2 hours under a constant 100 V. The PVDF membranes were blocked in 5% blocking buffer (5% skimmed milk in PBS) for 1 hour at room temperature. Blocked membranes were incubated over night with primary mouse monoclonal PDI antibody (ab2792) diluted in 5% blocking buffer at 1:1000 or β actin (ProteinTech-66009) diluted in 5% blocking buffer at 1:5000 in a cold room. On the following day, membranes were incubated with anti-mouse secondary antibody conjugated with horse-radish peroxidase (1:5000, GE Healthcare) for 1 hour and bands were observed by an enhanced chemiluminescence system.

2.4. Hormonal treatment in Ishikawa cells

Ishikawa cells expressing estrogen receptor (ER) and progesterone receptor (PR) were used. Ishikawa cells were treated with 5% charcoal dextran stripped-FBS (csFBS, Hyclone) with

phenol red-free MEM for 24 hours before treating with estrogen (0, 10, and 100 nM) or progesterone (0, 0.1, and 1 μM). After 24 hours, total RNA was extracted from the cells and real-time PCR was performed.

2.5. RNA extraction, reverse transcription and real-time PCR

Total RNA was extracted from the human endometrial samples using the MirVANA PARIS Kit (Invitrogen). TaqMan reverse transcription reagents and TaqMan 2X Universal PCR Master Mix (Life Technologies) were used to reverse transcribe the RNA samples to cDNA for the real-time PCR analysis. Real-time PCR was carried out in a QuantStudio 5 real-time PCR System (Applied Biosystems). TaqMan probes for human PDIA1 (Hs001050257_m1), PDIA2 (Hs00429010_m1), PDIA3 (Hs00607126_m1), PDIA4 (Hs01115905_m1), PDIA5 (Hs00895698_m1), and PDIA6 (Hs01012543_m1) were used to study the transcript expressions in the samples, with 18S (4318839) as the internal control. The 2-ΔΔCt method was used to calculate the relative mRNA expressions.

2.6. Spheroid-endometrial cells co-culture assay

Adhesion of Jeg-3 spheroids onto endometrial epithelial cells was quantified as reported previously [24, 27, 28]. Briefly, multi-cellular Jeg-3 spheroids were generated by shaking (4x g) the trypsinized Jeg-3 cells overnight. Spheroids with sizes between 60 and 200 μm were transferred on confluent monolayers of untreated/treated Ishikawa, RL95-2, AN3CA, or HEC1-B cells and incubated for 1 or 2 hours at 37°C in a 5% CO_2 humidified environment. Unattached spheroids were removed by shaking (8x g) the plate for 10 minutes. To study the effect of PDI antibody (PDI-RL77, ab5484) on the receptivity of endometrial cells,

AN3CA cells were pre-treated with the PDI antibody (5 or 10 µg/mL) and then washed and co-cultured with untreated Jeg-3 spheroids for 2 hours at 37°C in 5% CO_2. Similarly, bacitracin (PDI inhibitor, B0125-50KU, Sigma) was used to pretreat AN3CA and Ishikawa cells for 3 hours. Next, AN3CA cells were co-cultured with Jeg-3 spheroids for 2 hours at 37°C at 5% CO_2, while Ishikawa cells were co-cultured with Jeg-3 spheroids for 1 hour. The number of attached spheroids were counted under a light microscope and the attachment rate (% adhesion) was expressed as the number of attached spheroids over the total number of added spheroids as a percentage, as described previously [27-29].

2.7. Statistical analysis

Amino acids sequences were retrieved from the GenBank database and sequence alignment was performed using ClustalW (https://www.genome.jp/tools-bin/clustalw) and BoxShade (https://embnet.vital-it.ch/software/BOX_form.html). All numerical results were expressed as means ± SEM. Statistical comparisons were performed by t-test or one-way ANOVA using SPSS 20 and GraphPad Prism5. A $P<0.05$ was considered to be a significant difference. Each experiment was independently repeated at least three times.

3. Results

3.1. Sequence alignment of PDIA1-6 transcripts

We performed an amino acid sequence alignment of PDIA1-6 transcripts using ClustalW and BoxShade programs (Fig. 1A). We found that PDIA1-6 had some sequence homology, with overall amino acid similarity of less than 50% with the other PDIs. Interestingly, we found PDIA4 had a long stretch of N-terminal sequence, which was unique among the six PDI transcripts. Furthermore, PDIA1, PDIA3, PDIA4, PDIA5, and PDIA6 were found to be expressed in endocrine tissues, the pancreas, the gastrointestinal tract, the lungs, the brain, muscle tissues, lymphoid tissues, male reproductive tissues, and female reproductive tissues (endometrium, ovary, fallopian tube, and cervix), whereas PDIA2 was largely expressed in the pancreas, the gastrointestinal tract and, to a lesser extent, in female reproductive tissues such as the endometrium [30-32].

3.2. Expression of PDIA1-6 transcripts in endometrial epithelial cell lines

We studied the expression of PDIA1-6 transcripts in receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) human endometrial epithelial cells by qPCR. All four cell lines expressed PDIA1-6 transcripts (Fig. 1B). Among the four cell lines, HEC1-B cells had a significantly lower expression of PDIA1. Interestingly, non-receptive AN3CA and HEC1-B cells expressed significantly higher levels of PDIA2 compared with receptive Ishikawa and RL95-2 cells. No significant difference was found in the expression of PDIA3, PDIA4, and PDIA6 transcripts among the four cell lines. However, HEC1-B cells were found to have a significantly higher PDIA5 expression compared with Ishikawa, RL95-2, and AN3CA cells.

3.3. Expression of total, membrane, and cytoplasmic PDI proteins in endometrial epithelial cell lines

We further investigated the cellular distribution of PDI proteins in receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) human endometrial epithelial cells by Western blotting (Fig 1C). The membrane and cytoplasmic fractions of the proteins were isolated and subjected to Western blot analysis using antibodies against the PDI proteins. We found similar expression of total PDI proteins in all four cell lines. However, non-receptive cells (AN3CA and HEC1-B) had significantly higher amounts of membrane but lower amounts of cytoplasmic PDI proteins compared with receptive cells (Ishikawa and RL95-2). The purity of membrane and cytoplasmic fractions was confirmed by the presence of β-actin protein in cytoplasmic fraction and total β-actin protein as the control for protein loading.

3.4. Effect of steroid hormones on the expression of PDIA1-6 transcripts in Ishikawa cells

We next investigated the effect of estrogen and progesterone on the expression of PDI transcripts. As both estrogen and progesterone function through ligand-receptor binding, we studied the expressions of estrogen receptor alpha (ESR1) and progesterone receptor (PGR) in the four cell lines by qPCR (Suppl. Fig. 1). Receptive cells (Ishikawa and RL95-2) expressed ESR1 and PGR, whereas non-receptive cells (AN3CA and HEC1-B) expressed low or undetectable levels of both receptors. Therefore, we further investigated the effects of steroid hormones on the expression of PDIA1-6 in receptive Ishikawa cells expressing both ESR1 and PGR.

The addition of estrogen at 10 or 100 nM for 24 hours significantly induced the expression of PDIA1 and PDIA6 transcripts, but significantly suppressed transcript expression of PDIA4 (at

100 nM) and PDIA5 (at 10 and 100nM) when compared with the 0.1% ethanol (solvent) control (Fig 2A). Estrogen treatment at 10 and 100 nM had no effect on the expression of PDIA2 and PDIA3 transcripts in Ishikawa cells. Interestingly, progesterone treatment at both 0.1 and 1 μM for 24 hours suppressed the expression of PDIA1-6 transcripts in Ishikawa cells (Fig. 2B) when compared with the 0.1% ethanol (solvent) control.

3.5. Effect of PDI, PDI inhibitor, and PDI antibody on spheroid-endometrial cells co-culture assay

The in vitro spheroid-endometrial cells co-culture assay was used to study whether the expression of PDI proteins plays any role on spheroid attachment. We used trophoblastic Jeg-3 spheroids with sizes between 60 and 200 μm to compare the spheroid attachment rates on receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) epithelial cells (Fig. 3A). In each attachment assay, 30 spheroids were added on top of a monolayer (Fig. 3A) and the attachment rate after 1 and 2 hours of co-culture was recorded. We found the attachment rates after 1 and 2 hours were highest on Ishikawa cells (87.4% and 93.1%) followed by RL95-2 (84% and 86.2%), and then HEC1-B (11.4% and 31.3%) and AN3CA (4% and 26.2%).

To study the functional role of PDIs on the receptivity of endometrial cells, non-receptive AN3CA cells that express high levels of membrane PDI proteins were investigated. The AN3CA cells were treated with 0.5, 1, and 5 mM PDI inhibitor (bacitracin) for 24 hours. The attachment rate of Jeg-3 spheroids increased between 13% and 17% in treated AN3CA cells compared with untreated AN3CA cells (Fig. 3B). Similarly, AN3CA cells were treated with 5 or 10 μg/mL of PDI antibody for 24 hours. The attachment rate of Jeg-3 spheroid increased by 12% and 10% in the treated AN3CA cells, respectively, compared with untreated AN3CA

cells (Fig. 3B). No observable change in the attachment rate was found when a control antibody was used at 2 μg/mL. On the contrary, receptive Ishikawa cells expressing lower levels of membrane PDIs treated with 5 mM bacitracin did not further increase the attachment rate of Jeg-3 spheroids (Fig 3C). To exclude the possibility that bacitracin altered the expression of PDI transcripts in the treated AN3CA cells, we performed a qPCR analysis of PDIA1-6 transcripts, which found no significant changes in their transcript expressions after bacitracin treatment (Fig 3D).

4. Discussion

Endometrial receptivity is regulated by various factors including the expression of membrane proteins that facilitate or block embryo attachment. Here, we have reported that PDIs in endometrial epithelial cells play an important role in the implantation process. The expressions of PDI transcripts were downregulated by progesterone in receptive Ishikawa cells. High levels of membrane PDI proteins were found in non-receptive AN3CA and HEC1-B cells. The receptivity of AN3CA to Jeg-3 spheroid attachment increased with pre-treatment with PDI inhibitor or PDI antibody, suggesting the membrane expression of PDI proteins may modulate embryo attachment in vivo.

We used mass spectrometric analysis to show differential expressions of PDIA1 membrane proteins in receptive endometrial cells when compared with the other cell lines (unpublished data). The PDI family of proteins consist of 21 members, with PDIA1-6 the most studied in various human tissues [33-34]. The amino acid sequence alignment of PDIA1-6 indicated low similarity between these PDI proteins. We investigated the expression of PDIA1-6 transcripts in receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) cell lines. Although we found similar expressions of PDI proteins, the expression levels of membrane PDI proteins were higher in non-receptive endometrial cells (AN3CA and HEC1-B). The reasons for the higher expression levels of membrane PDI proteins in non-receptive cells remain unknown, but a steroid hormone regulatory mechanism may be involved that shuffles between cytoplasmic and membrane proteins [35-36]. For example, it has been reported that estrogen induces E cadherin translocation via a clathrin-dependent pathway in HC11-siERβ cells via ligand activation of ERα [37]. In our study, we also found non-receptive endometrial

cells (AN3CA and HEC1-B) expressed low or undetectable levels of steroid receptors ESR1 and PGR, suggesting that PDI shuffling may be hampered in non-receptive endometrial cells.

Estrogen treatment at 100 nM in Ishikawa cells upregulated transcript expression of PDIA1 and PDIA6, but downregulated PDIA4 and PDIA5. No significant expression changes in PDIA2 and PDIA3 were observed in estrogen-treated Ishikawa cells. Progesterone was found to downregulate the expression of PDIA1-6 in the treated Ishikawa cells. In line with this, bovine aortic endothelial cells were reported to have upregulated PDI upon estrogen treatment [38]. Further studies are needed to investigate the combined effects of steroid hormones on the expression of PDI transcripts to mimic steroid hormone changes in vivo.

PDI regulates the expression of other implantation associated molecules such as integrin β3, E-cadherin, and EGFR. PDI binds to integrin β3 in regulating endothelial and platelet functions, and significantly lower endometrial integrin β3 expression is observed in repeated implantation failure patients [39]. A recent study reported that PDIA1 silencing increased E-cadherin expression in HKE3 cells [40]. A significant reduction in endometrial luminal E-cadherin expression was reported in infertile women compared with fertile women [41]. Downregulation of EGFR could significantly reduce receptivity of RL95-2 cells [42], and upregulate PDIA6 in U87MG cells [43], suggesting the collective role of PDIA6 in regulating endometrial receptivity with other implantation associated molecules.

Due to limitations in the availability of human primary endometrial epithelial cells and human embryos for the research purposes, we used receptive (Ishikawa and RL95-2) and non-receptive (AN3Ca and HEC1-B) endometrial epithelial cells to perform the attachment assays using trophoblastic Jeg-3 spheroids. In this study, we observed a higher Jeg-3 spheroid attachment rate on receptive endometrial cells (Ishikawa and RL95-2) compared with non-

295 receptive cells (AN3CA and HEC1-B). These results agreed with previous studies that used different endometrial cell lines in the attachment assays [23, 24].

We further investigated the association between endometrial receptivity and PDI functions. Two approaches were used to study the role of PDIs in endometrial receptivity via the spheroid attachment assay. First, we used the PDI inhibitor bacitracin, which functions to 300 inhibit the reductive activity of PDI [44], but does not alter the expression PDI proteins [45]. Although it has been reported that bacitracin is non-specific for PDI inhibition [46], our data indicated that bacitracin enhanced Jeg-3 spheroid attachment onto treated AN3CA cells when compared with the control, suggesting that inhibiting PDI function enhances spheroid attachment. Second, we used PDI antibody to treat AN3CA cells before the spheroid 305 attachment assay. Similarly, the findings suggest that blocking PDI protein enhances spheroid attachment. Many previous studies have also reported that functional blocking of cell surface proteins by antibodies affected spheroid attachment. For example, JAr spheroid attachment on HEC1-A cells was increased after cell surface blockage of TUBB2C, whereas JAr spheroid attachment on RL95-2 cells was reduced with anti-CRT antibody/HSPA9 antibody treatment 310 [22]. Conversely, receptive endometrial epithelial Ishikawa cells treated with PDI inhibitor did not further enhance spheroid attachment, suggesting that lower expression of membrane PDI favors spheroid attachment.

In summary, a higher expression of membrane PDI regulated by estrogen and progesterone in the endometrial epithelium creates an environment that is non-conducive 315 for healthy embryo implantation. Further studies on the role of estrogen and progesterone receptors in steroid hormone-mediated membrane PDI expression may provide valuable

insights on the role of PDI transcripts on endometrial receptivity and early embryo attachment.

Declaration of Conflicts of Interest

The authors have no conflicts of interest to declare.

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Figure Legends

Figure 1 Human protein disulfide isomerase (PDI) sequence and expression in endometrial cells. (A) Alignment of PDIA1 (P07237), PDIA2 (Q13087), PDIA3 (P30101), PDIA4 (P13667), PDIA5 (Q14554), and PDIA6 (Q15084) amino acid sequences with ClustalW and BoxShade. Conserved sequences are shown on the bottom. (B) Expression of PDIA1-6 transcripts in receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) human endometrial epithelial cells. N=5, $p<0.05$. (C) Expression of total, membrane, and cellular PDI proteins in the four cell lines. The expression of β-actin was used as a loading control and for purity of the different cellular fractions.

Figure 2 Effect of estrogen and progesterone on the expression of PDI isoforms in Ishikawa cells. The effect of (A) estrogen and (B) progesterone on the expression of PDIA1-6 transcripts. Ishikawa cells were treated with estrogen (10 and 100 nM) or progesterone (0.1 and 1 μM) for 24 h, and total RNA was extracted for real-time RT-PCR analysis using TaqMan probes specific for PDIA1-6 transcripts. Ethanol at 0.1% was used as the control. N=4-5, $p<0.05$.

Figure 3 Role of PDI on spheroid attachment on human endometrial epithelial cells. (A) Photomicrograph of spheroids attached to receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) endometrial epithelial cells. Arrowheads indicate the attached Jeg-3 spheroids on the endometrial epithelial cells. The corresponding spheroid attachment rate after 1 and 2 h co-culture. The number of attached spheroids over total spheroids added

is shown in the bars. (B) Effect of PDI inhibitor (bacitracin) and PDI antibody on the attachment rate of Jeg-3 spheroids onto non-receptive AN3CA cells. PBS and control antibody were used as controls in the experiment, respectively. (C) Effect of PDI inhibitor (bacitracin) on the attachment rate of Jeg-3 spheroids onto receptive Ishikawa cells. PBS at 0.1% was used as a control. N=4-6, $p<0.05$. (D) Effect of bacitracin on the expression of PDIA1-6 transcripts in treated AN3CA cells. N=4, $p<0.05$.

Supplementary Figure 1 Expression of steroid receptors in human endometrial epithelial cells. Real-time PCR was used to detect the expression of estrogen receptor alpha (ESR1) and progesterone receptor (PGR) in receptive (Ishikawa and RL95-2) and non-receptive (AN3CA and HEC1-B) cells. N=3, $p<0.05$.

Figure 1

A

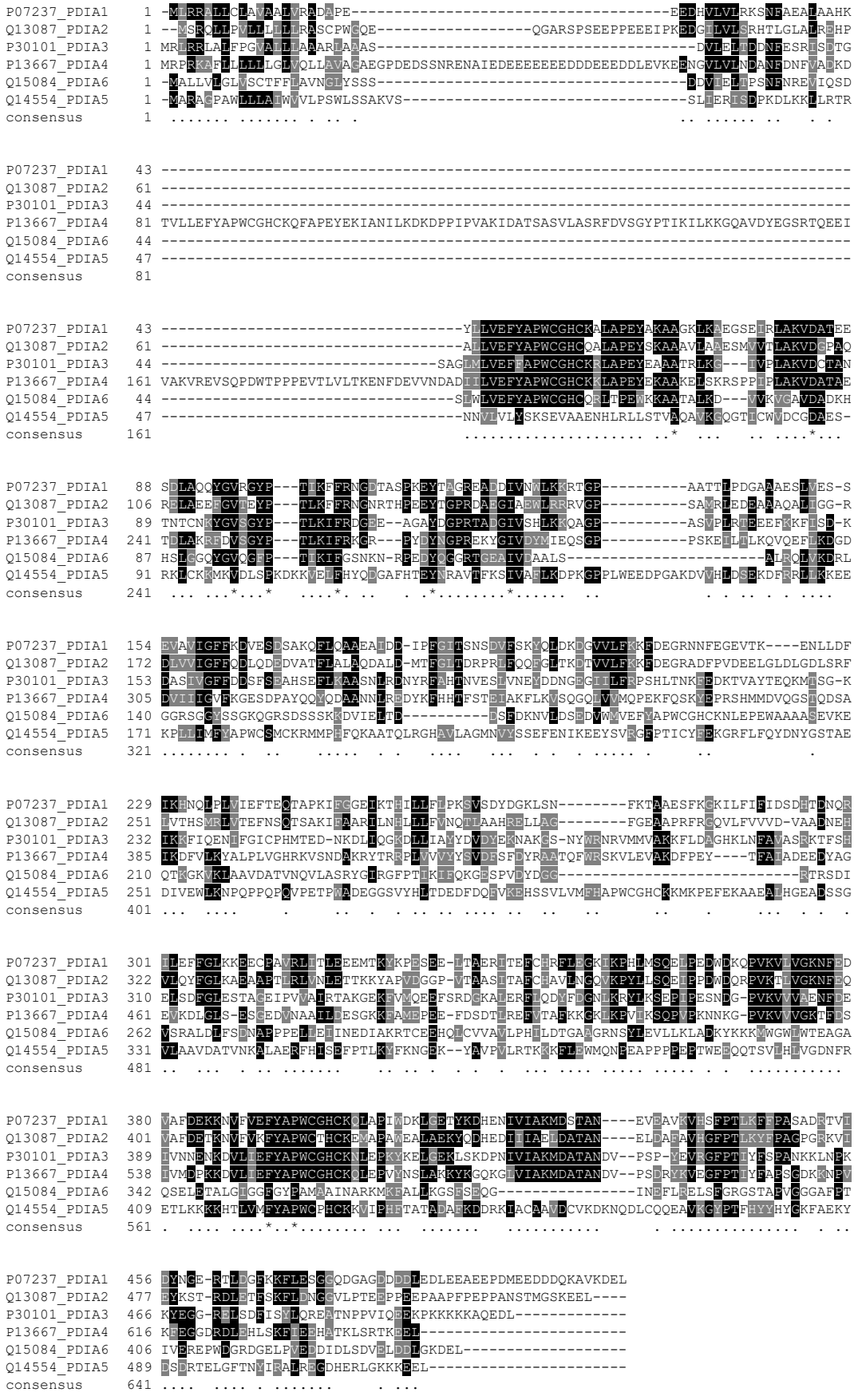
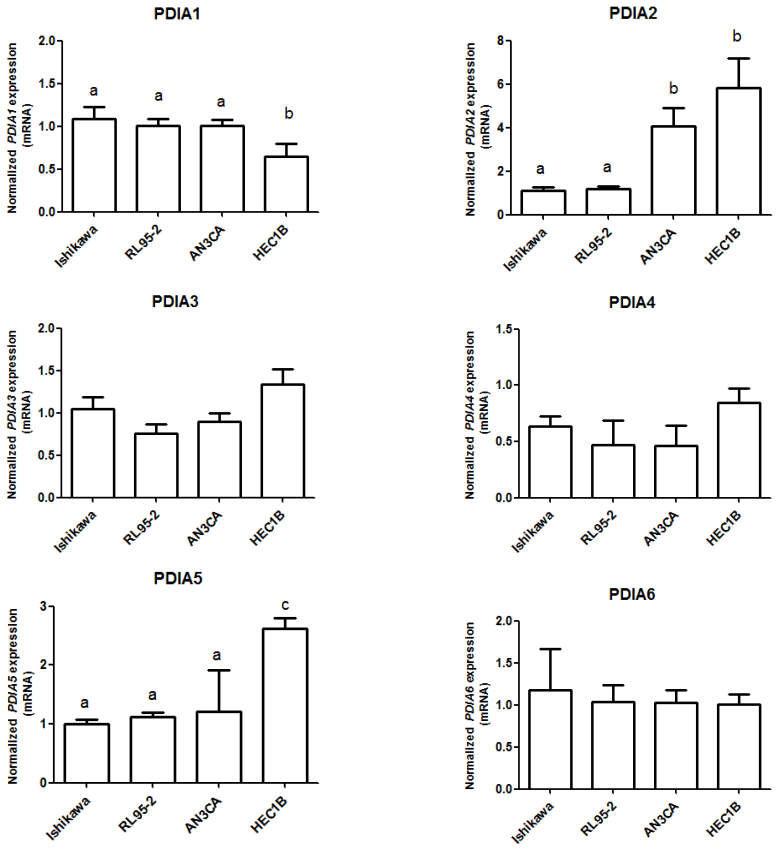

P07237_PDIA1
Q13087_PDIA2
P30101_PDIA3
P13667_PDIA4
Q15084_PDIA6
Q14554_PDIA5
consensus

Figure 1

B


PDIA1
Normalized PDIA1 expression (mRNA)
2.0
1.5
1.0
0.5
0.0
a
a
a
b
Ishikawa
RL95-2
AN3CA
HEC1B
PDIA2
Normalized PDIA2 expression (mRNA)
8
6
4
2
0
a
a
b
b
Ishikawa
RL95-2
AN3CA
HEC1B
PDIA3
Normalized PDIA3 expression (mRNA)
2.0
1.5
1.0
0.5
0.0
Ishikawa
RL95-2
AN3CA
HEC1B
PDIA4
Normalized PDIA4 expression (mRNA)
1.5
1.0
0.5
0.0
Ishikawa
RL95-2
AN3CA
HEC1B
PDIA5
Normalized PDIA5 expression (mRNA)
3
2
1
0
a
a
a
c
Ishikawa
RL95-2
AN3CA
HEC1B
PDIA6
Normalized PDIA6 expression (mRNA)
2.0
1.5
1.0
0.5
0.0
Ishikawa
RL95-2
AN3CA
HEC1B

C

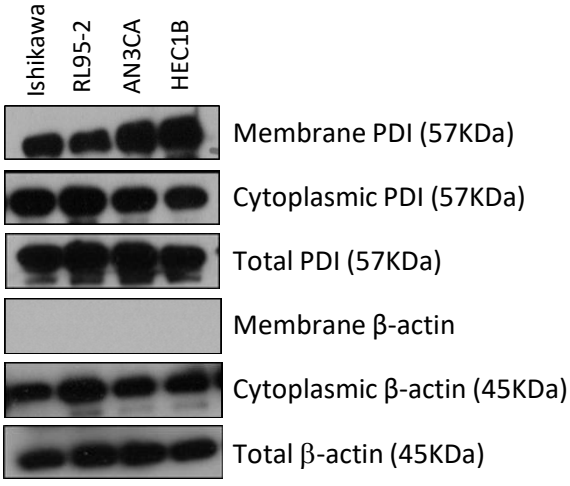
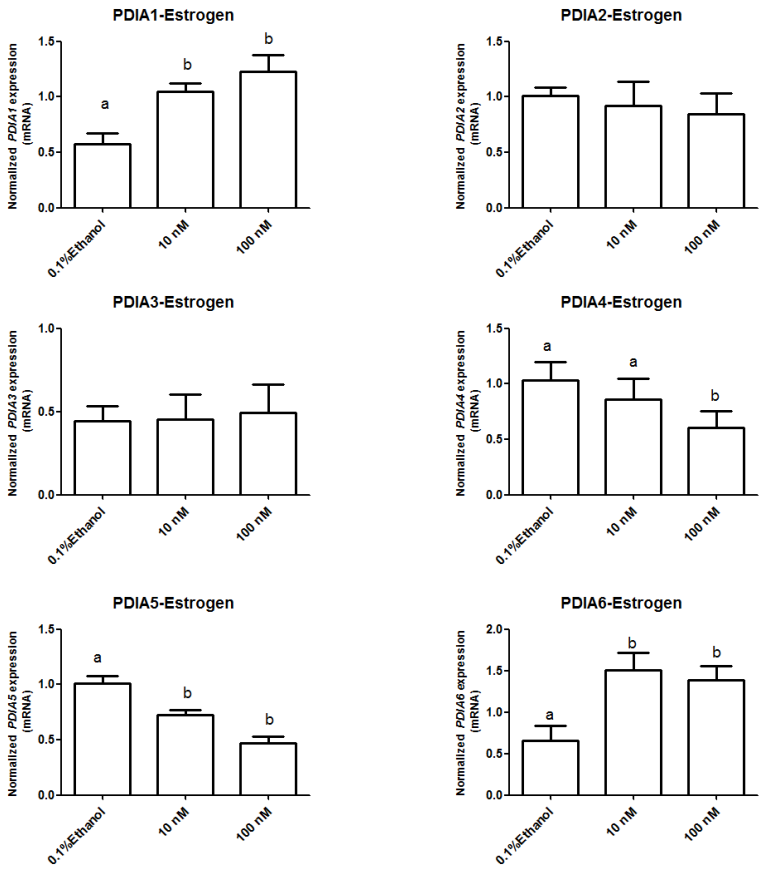

Ishikawa
RL95-2
AN3CA
HEC1B
Membrane PDI (57KDa)
Cytoplasmic PDI (57KDa)
Total PDI (57KDa)
Membrane β-actin
Cytoplasmic β-actin (45KDa)
Total β-actin (45KDa)

Figure 2

A


PDIA1-Estrogen
Normalized PDIA1 expression (mRNA)
a
b
b
PDIA2-Estrogen
Normalized PDIA2 expression (mRNA)
PDIA3-Estrogen
Normalized PDIA3 expression (mRNA)
PDIA4-Estrogen
Normalized PDIA4 expression (mRNA)
a
a
b
PDIA5-Estrogen
Normalized PDIA5 expression (mRNA)
a
b
b
PDIA6-Estrogen
Normalized PDIA6 expression (mRNA)
a
b
b
0.1%Ethanol
10 nM
100 nM

B

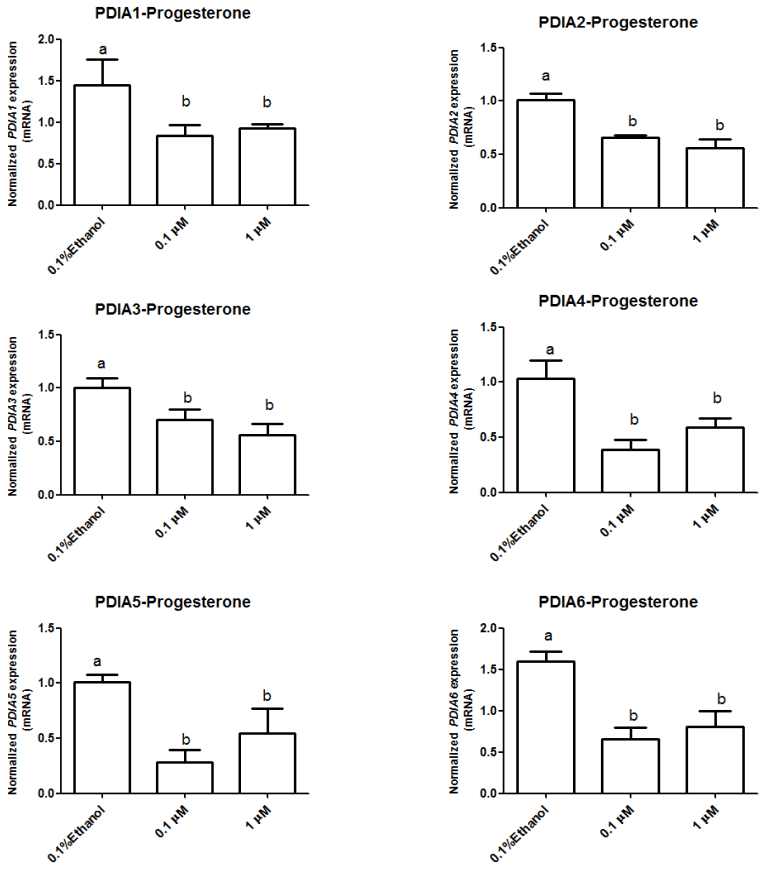
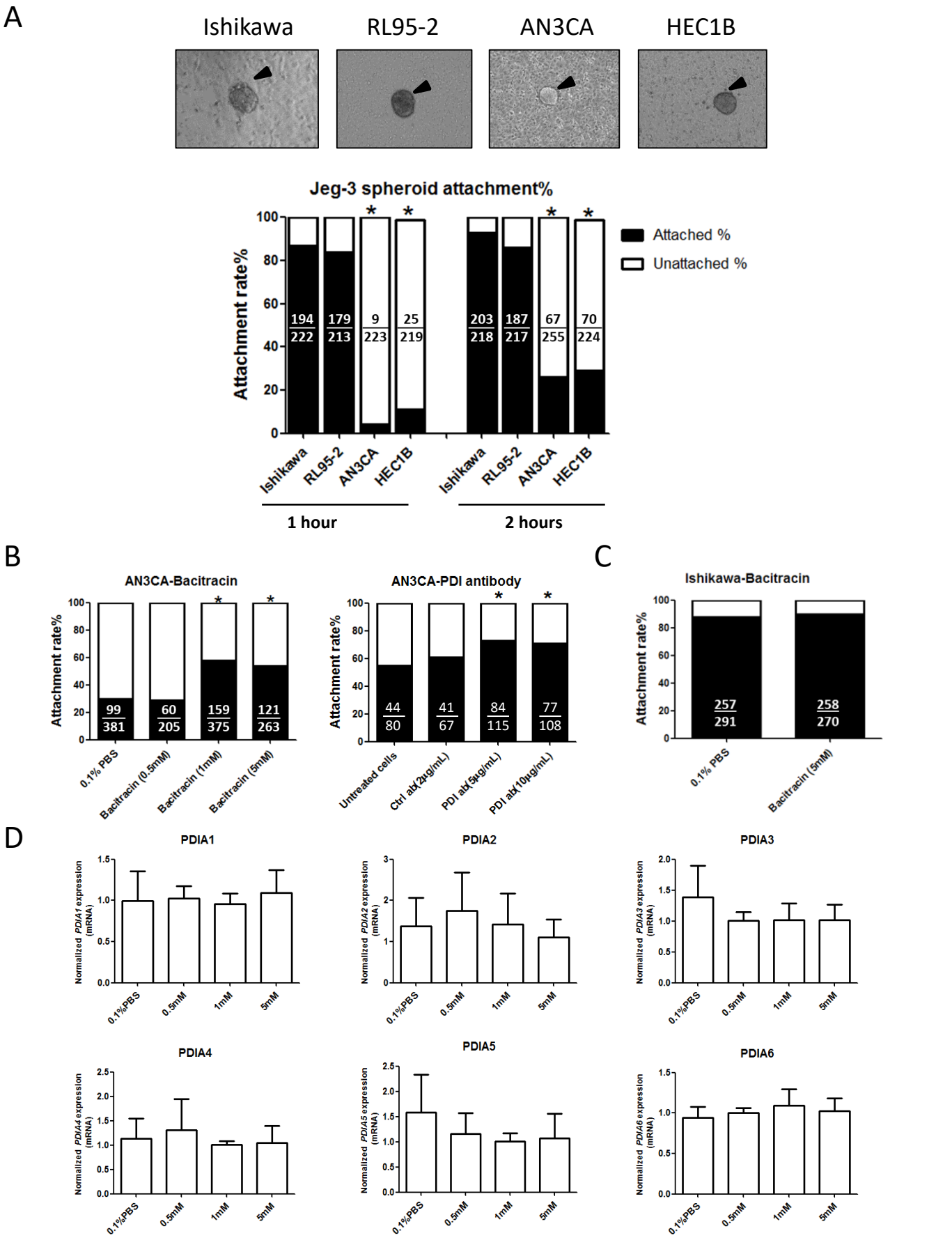

PDIA1-Progesterone
Normalized PDIA1 expression (mRNA)
a
b
b
PDIA2-Progesterone
Normalized PDIA2 expression (mRNA)
a
b
b
PDIA3-Progesterone
Normalized PDIA3 expression (mRNA)
a
b
b
PDIA4-Progesterone
Normalized PDIA4 expression (mRNA)
a
b
b
PDIA5-Progesterone
Normalized PDIA5 expression (mRNA)
a
b
b
PDIA6-Progesterone
Normalized PDIA6 expression (mRNA)
a
b
b
0.1%Ethanol
0.1 μM
1 μM

Figure 3


A
Ishikawa
RL95-2
AN3CA
HEC1B
Jeg-3 spheroid attachment%
Attachment rate%
Attached %
Unattached %
194/222
179/213
9/223
25/219
203/218
187/217
67/255
70/224
Ishikawa
RL95-2
AN3CA
HEC1B
1 hour
2 hours
B
AN3CA-Bacitracin
99/381
60/205
159/375
121/263
0.1% PBS
Bacitracin (0.5mM)
Bacitracin (1mM)
Bacitracin (5mM)
AN3CA-PDI antibody
44/80
41/67
84/115
77/108
Untreated cells
Ctrl ab(2μg/mL)
PDI ab(5μg/mL)
PDI ab(10μg/mL)
C
Ishikawa-Bacitracin
257/291
258/270
0.1% PBS
Bacitracin (5mM)
D
PDIA1
PDIA2
PDIA3
PDIA4
PDIA5
PDIA6
Normalized PDIA1 expression (mRNA)
Normalized PDIA2 expression (mRNA)
Normalized PDIA3 expression (mRNA)
Normalized PDIA4 expression (mRNA)
Normalized PDIA5 expression (mRNA)
Normalized PDIA6 expression (mRNA)
0.1%PBS
0.5mM
1mM
5mM

Suppl Figure 1