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Role of endometrial estrogen & progesterone receptors on Protein Disulphide Isomerase1(PDIA1) expression on regulating endometrial receptivity

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Introduction

Differentially expressed surface proteins at the receptive phase of endometrial epithelium play a pivotal role on embryo implantation. Protein Disulphide Isomerase (PDI) family contains 21 members and PDI function as chaperone protein with redox activity. Recent studies suggested a differential expression of PDI on cell surface of endometrial epithelial cells RL95-2 and HEC-1A

Study Question

Do steroids regulate PDIA1 expression in endometrium via steroid receptors which in turn govern the endometrial receptivity?

Aims

- To study PDIA1 expression in total protein & membrane protein of endometrial epithelial cell lines
- To study the steroid regulation of PDIA1 expression in endometrial cells via steroid receptors
- To study the functional role of PDIA1 on spheroid attachment

Methodology

We used receptive (Ishikawa and RL95-2) and non-receptive (HEC1-B and AN3CA) human endometrial cells lines and Jeg-3 trophoblastic cell line for co-culture study. Total protein, membrane protein and cytosolic protein were extracted and specific antibodies were used to analyze the enrichment of proteins in different fractions. The expressions of PDIA1 were studied by Western blotting and immunohistochemistry.

Results

1. Membrane PDIA1 expression is high in AN3CA cells

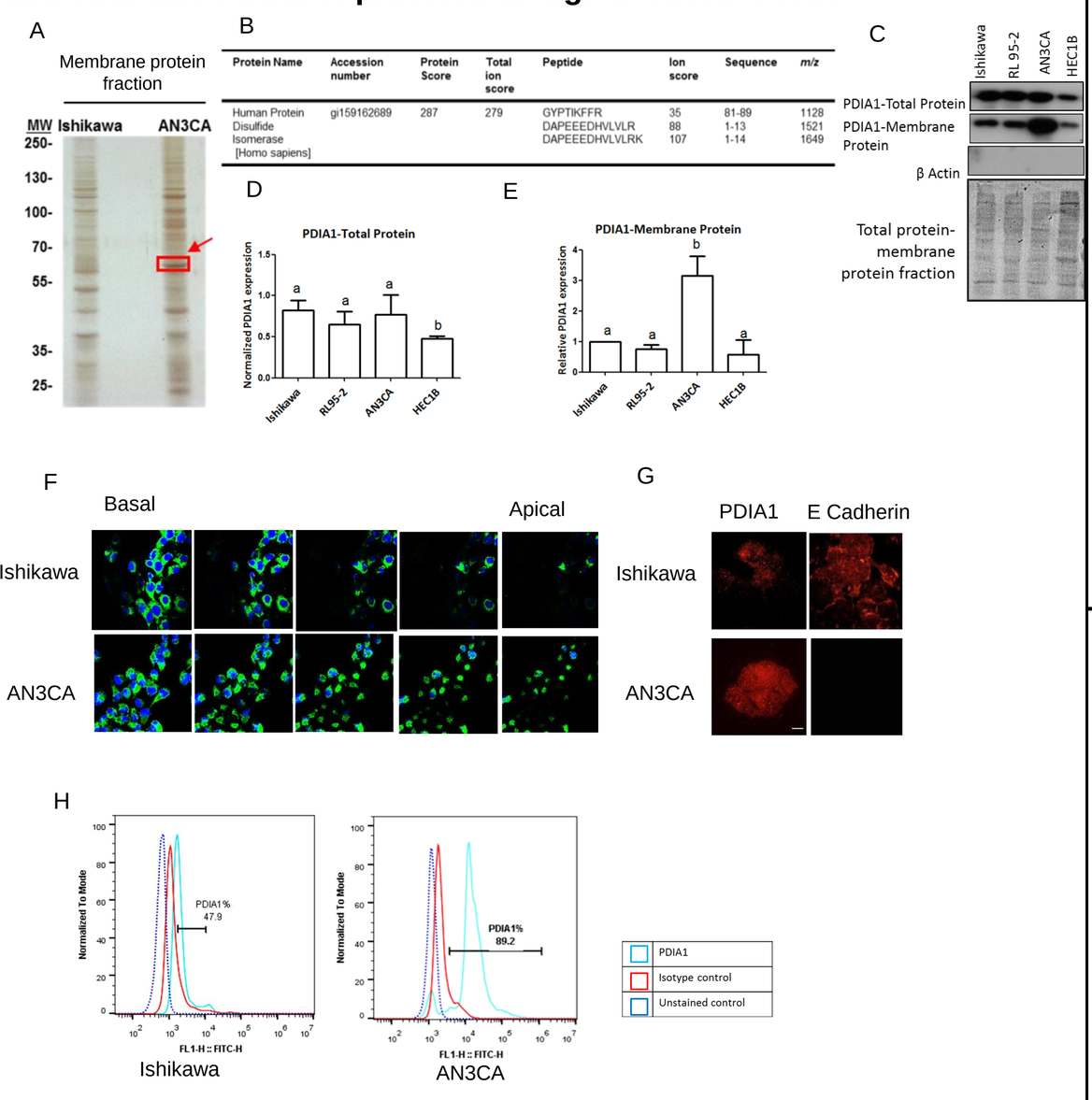


Fig. 1 Expression of PDIA1 protein in 4 endometrial epithelial cell lines (Ishikawa, RL95-2, AN3CA & HEC1B). (A) Identification of PDI in endometrial epithelial cells by mas spectrometry. Silver staining of endometrial cell lines Ishikawa & AN3CA separated by SDS-PAGE. The red rectangle marks the band subjected for mass spectrometry analysis. (B) The protein was identified as human PDI. (C,D,E) Western blotting confirmed PDIA1 protein were highly abundant in membrane protein of AN3CA compared to other cell lines (N=5). *denotes significant difference among the groups $p<0.05$.(F) Immunofluorescence staining for PDIA1 in Ishikawa (Receptive) & AN3CA (Non-receptive) cell lines at different z-sections shows abundance of PDIA1 in apical region of AN3CA (G) TIRF microscopy images further shows the abundance of fluorescence signals on AN3CA cells. E-cadherin was used as a membrane marker in Ishikawa cells, scale bar-10μm(H), Flow cytometry for cell surface shows abundance of cell surface PDIA1 in AN3CA cells

2. Estrogen up regulates PDI expression in Ishikawa cells, but progesterone down regulates PDI expression

I. Estrogen up regulates, Progesterone down regulates PDI in total & membrane protein and Estrogen & Progesterone down regulates membrane PDIA1

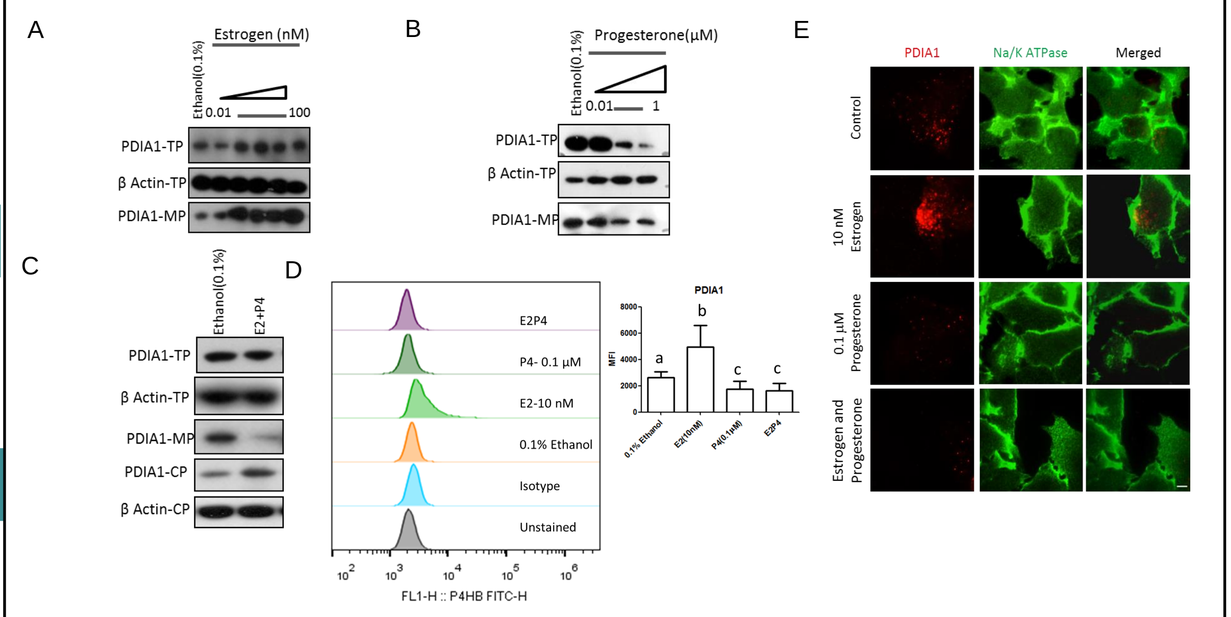


Fig. 2 (A) PDIA1 Is significantly up regulated by estrogen at 0.1-100nM in total(TP) & membrane(MP) protein. (B) PDIA1 is significantly down regulated in TP & MP by progesterone at 0.1-1uM (C) Total PDIA1 is not changed, membrane PDIA1 Is down regulated & cytosolic PDIA1 is increased by estrogen & progesterone suggesting localization difference, Flow cytometric analysis of cell surface PDIA1(D) & TIRF images (E) shows up regulation of membrane PDIA1 by estrogen and down regulation by progesterone/estrogen & progesterone, MFI- Mean Fluorescence Intensity * $p<0.05$,scale bar-10μm

III. PDIA1 expression at different phases of human menstrual cycle

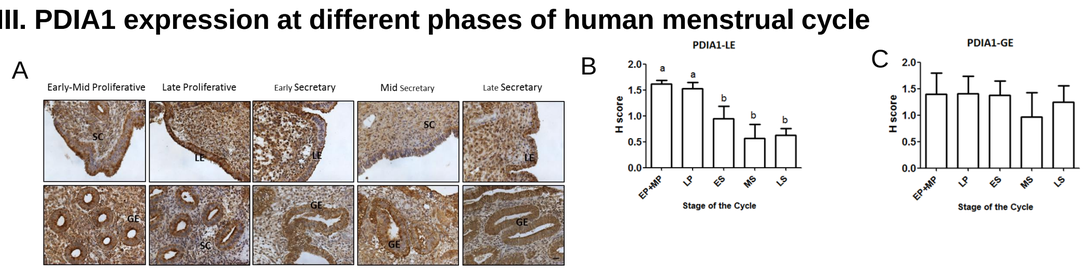


Fig. 3 (A) PDIA1 expression in phases of human menstrual cycle, Apical PDIA1 expression is significantly high in proliferative phase of luminal epithelium-LE (B) than secretary phase and no significant change in glandular epithelium-GE among the phases (C), Scale bar = 20μm

3. Steroid receptors regulate PDIA1 expression

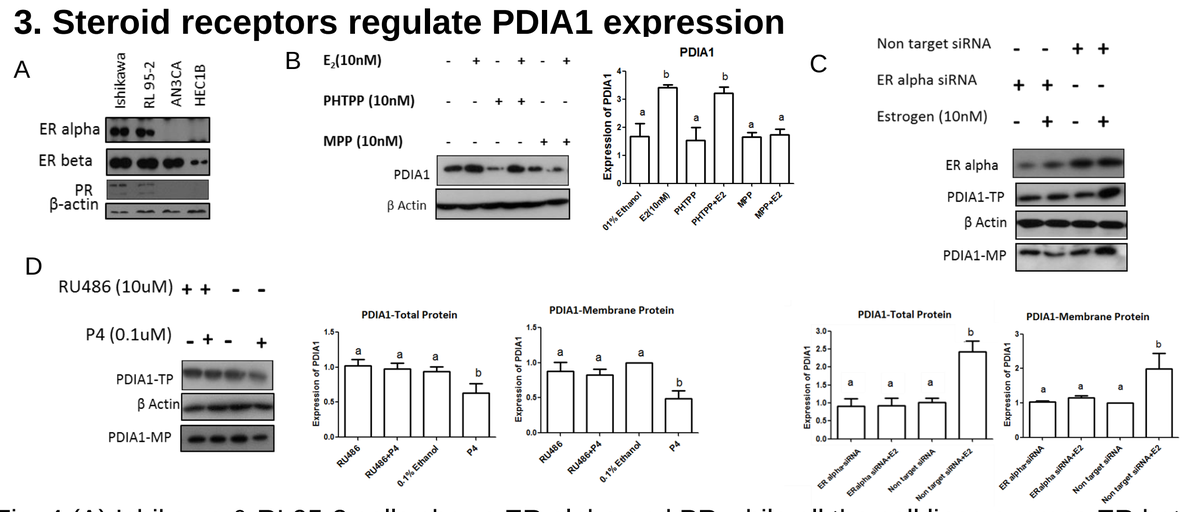


Fig. 4 (A) Ishikawa & RL95-2 cells shows ER alpha and PR while all the cell lines express ER beta. (B) MPP-ER alpha antagonist inhibits estrogen mediated PDIA1 up regulation, while no change in PHTPP-ER beta antagonist treated cells (C) ER alpha knock down, inhibits estrogen mediated PDIA1 up regulation, (D) PR antagonist-RU486 inhibits progesterone mediated PDIA1 down regulation * $p<0.05$

4. PDIA1 regulates spheroid attachment

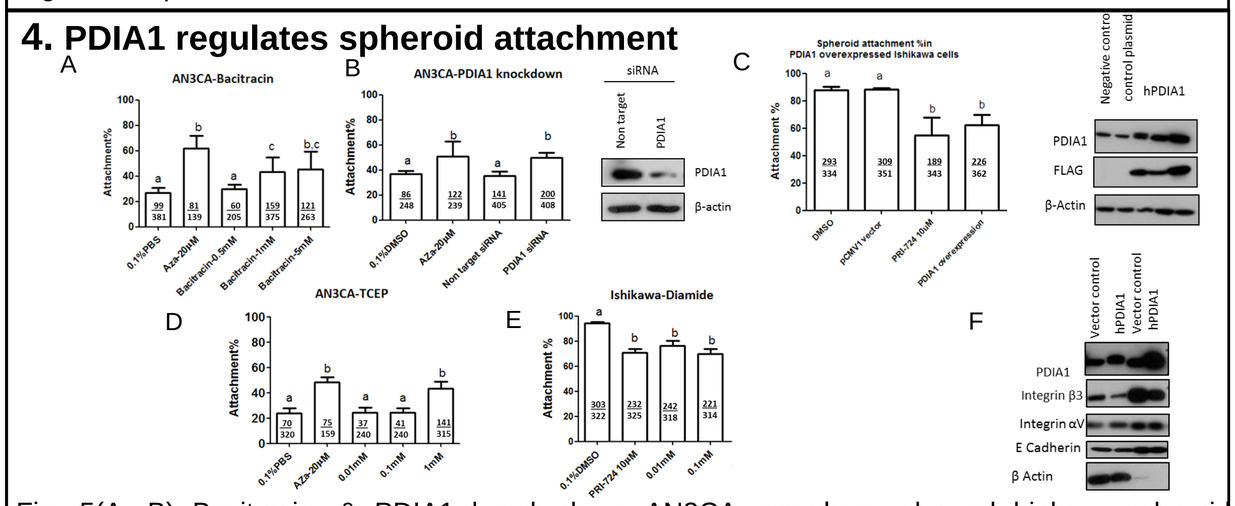
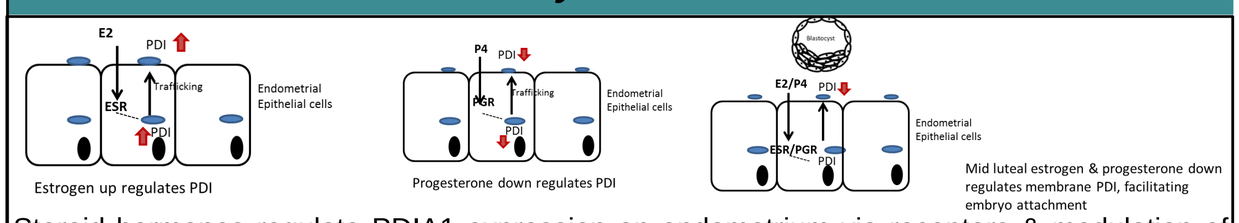


Fig. 5(A, B) Bacitracin, & PDIA1 knock down AN3CA monolayer showed higher spheroid attachment rate, (C) PDIA1 over expressed Ishikawa monolayer showed reduced attachment, (D) TCEP increased attachment on AN3CA (E) Diamide reduced attachment on Ishikawa (F) Integrin beta3 is down regulated in PDIA1 over expressed cells, no change in E cad or integrin alphaV (*$p<0.05$).

Summary & Conclusion



Steroid hormones regulate PDIA1 expression on endometrium via receptors & modulation of PDI function with inhibitors may provide a new approach to enhance implantation rate

Acknowledgement : The work is supported by the HMRF grant 15162211 to KF Lee