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Abstract title:

Non-classical progesterone signaling may be sufficient to induce decidualization in Human Endometrial Stromal Cells (ESC)

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Study question:

Is it progesterone (P4) entry into the human endometrial stromal cells (ESC) necessary to produce a correct decidualization?

Summary answer:
P4 entry in ESC is not necessary to induce in vitro decidualization as long as PGRMC1 is inhibited by AG205.

What is known already:
PGRMC1 is a membrane receptor that mediates non-genomic action of P4.It has been implicated in a diversity of biological events,including apoptosis and folliculogenesis(1,2).PGRMC1 mediates non-classical P4 signaling in endometrium but its mechanism of action is still unknown.PGRMC1 protein is down-regulated in receptive endometrium but its expression is higher in stromal compartment compared to epithelial cells(3).We previously published that it localizes in membrane, cytosol and nucleus of non-decidualized ESC,but accumulates in the nucleus of decidualized ESC (dESC).Its overexpression inhibits in vitro decidualization,co-localizing with SERPINE 1 mRNA binding protein (SERBP1) throughout the menstrual cycle and in the cytosol of non-decidualized and decidualized ESC (4).

Study design, size, duration:
Endometrial biopsies were obtained from donors (n=7) and ESC were isolated. In vitro decidualization was induced by a long protocol using P4/E2 (Estradiol) (8 days) and a short protocol using cyclic adenosine monophosphate/Medroxyprogesterone (cAMP/MPA) (4 days). To activate only membrane progesterone receptors we used a membrane-impermeable P4 (P4-BSA) that avoids P4 entry in the cell. Also, we employed AG205, a PGRMC1 antagonist that blocks PGRMC1 activation.

Participants/materials, setting, methods:
We induced in vitro decidualization by long protocol with P4(1uM)/E2 (10nM) or P4-BSA(1uM)/E2 in combination with AG205 (50uM) (PGRMC1 antagonist), and by short protocol with MPA/cAMP or P4-BSA/cAMP+AG205. Decidualization was checked by IGFBP1 and Prolactin (PRL) secretion by ELISA and F-actin staining with phalloidin in each condition (n=7). Classic progesterone receptor (PRAB) and PGRMC1 localization was performed in non-decidualized ESC (ndESC) and decidualized ESC (dESC) of both protocols by immunofluorescence (n=4).

Main results and the role of chance:
PRL secretion significantly decreased in the presence of P4-BSA/E2 compared to dESC P4/E2 group ($p<0.05$). Nevertheless, PRL levels were significantly increased in the presence of P4-BSA/E2+AG205 compared to ndESC and dESC P4-BSA/E2 group ($p<0.05$). However, when we use the short protocol, all groups were able to secrete high levels of PRL included the P4-BSA/cAMP and P4-BSA/cAMP+AG205 groups. F-actin staining showed a lack of cytoskeleton reshaping in P4-BSA dESC group compared to the typical polygonal shape observed in decidualized controls. The F-actin filaments disposition of a

dESC were restored in P4-BSA group in the presence of AG205. Again, dESC of all groups showed a correct F-actin structure when we induced decidualization with the short protocol, also in the P4-BSA/cAMP and P4-BSA/cAMP +AG205 groups. Immunocytofluorescence assay revealed a clear PGRMC1 perinuclear and nuclear sublocalization in P4/E2 and P4/E2+AG205 dESC groups, while it persisted at the membrane and cytosol in P4-BSA/E2 and P4-BSA/E2+AG205 dESC groups. Surprisingly, PRAB, continued translocating to the nucleus in P4-BSA/E2 dESC group as in the control (P4/E2). However, PRAB signal strongly decreased in P4-BSA/E2+AG205 dESC group. Finally, immunocytofluorescence of PR receptors using the short protocol did not show localization differences between all the groups in both receptors.

Limitations, reasons for caution:
Due to the variability between human endometrial samples and the low number of experiments performed, results should be taken with caution.

REFERENCES
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Wider implications of the findings:
According to our results, we demonstrated that in absence of the P4 entry into the stromal cell, the activation of P4 membrane receptors in concomitance with PGRMC1 inhibition, is sufficient to achieve in vitro decidualization. Further molecular experiments are needed to understand the underlying mechanisms.

Trial registration number:
Not applicable.

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Keywords:
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