Regulation of *PGR* gene expression in immortalized human endometrial stromal cells

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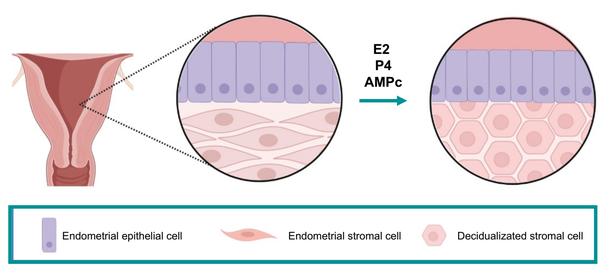
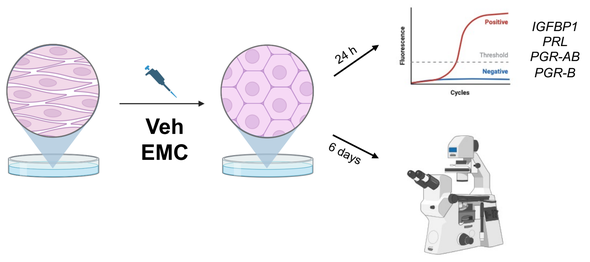
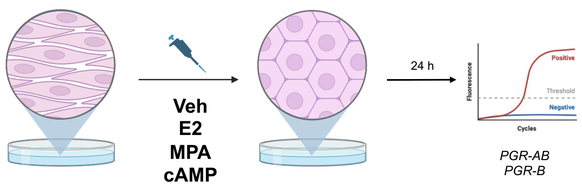
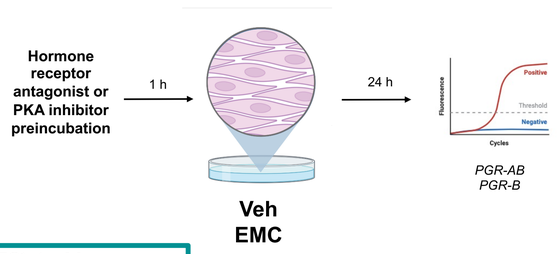
Background: Decidualization, a crucial process for successful pregnancy establishment and maintenance, involves endometrial stromal cell differentiation.[1] This process is orchestrated by estradiol (E2), progesterone, and other stimuli that increase intracellular cyclic adenosine monophosphate (cAMP) levels.[1] The progesterone receptor (PR) plays a pivotal role in regulating decidualization,[2] and alterations in its expression are linked to endometrial pathologies.[3] However, the mechanisms governing PR gene (*PGR*) expression in endometrial stromal cells (ESCs) during decidualization are not fully understood. This study aimed to identify the mechanisms of *PGR* expression regulation in immortalized human endometrial stromal cells.



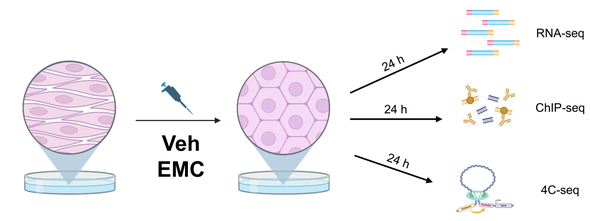
Methods:







10 nM 17β-estradiol (E2) 1 μM medroxyprogesterone (MPA) or progesterone (P4)+ 0.5 mM cAMP, 1 μM MPP dihidrochloride, 1 μM PHTPP, 1 μM ICI182,780, 1 μM Mifepristone (RU486), and 10 μM H89.



Results:

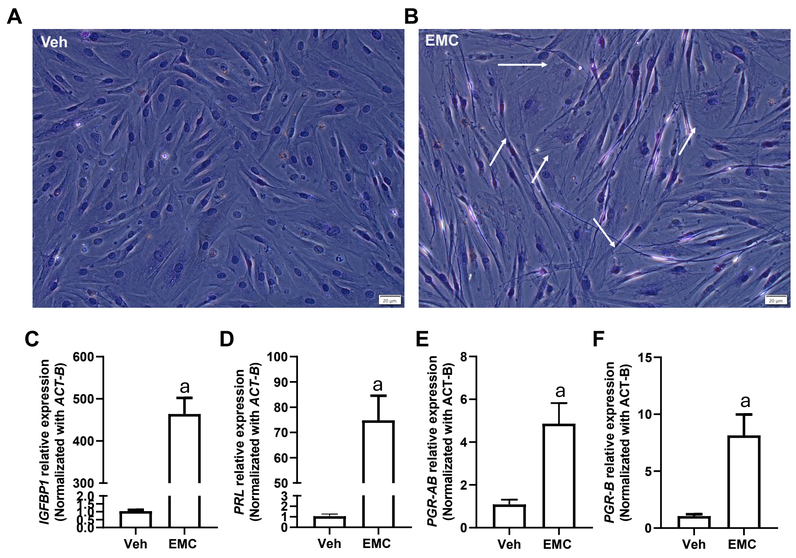


Figure 1. *In vitro* decidualization of T-HESCs. The micrographs show the fibroblast morphology of T-HESCs treated with ethanol (Veh, A) and the decidualized epithelioid morphology of these cells when treated with E2, MPA, and cAMP (EMC, B) for six days. Total RNA was isolated from T-HESCs treated with vehicle or EMC for 24 h. The RNA was subjected to RT-qPCR analysis to detect *IGFBP1* (C), *PRL* (D), *PGR-AB* (E), and *PGR-B* (F) expression. *ACT-B* was used as a reference, constitutively expressed gene. Values shown in the figures are means and SEM obtained from 3 independent cultures. **a**, P≤0.05 vs. vehicle (unpaired t-test).

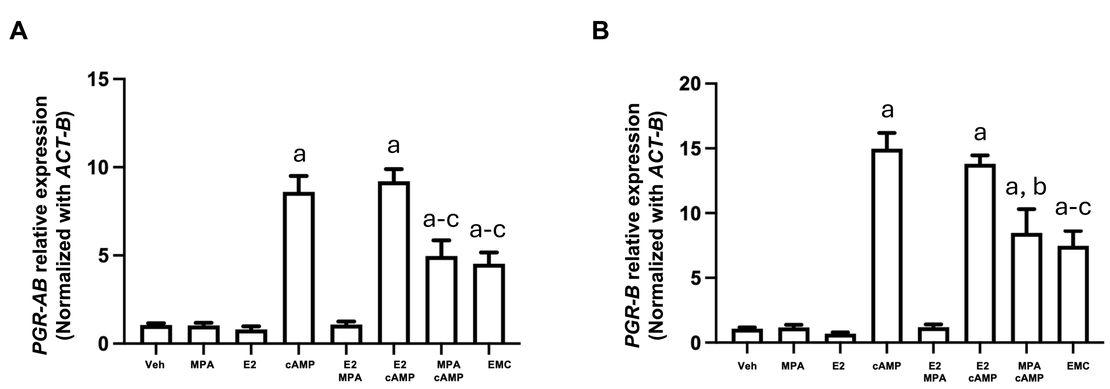


Figure 2. Regulation of *PGR isoforms* in T-HESCs with different stimuli involved *in vitro* decidualization. Total RNA was isolated from T-HESCs treated with vehicle, individually in each possible combination involved in decidual stimulus with E2, MPA, and cAMP (EMC) at 24 h. The RNA was subjected to RT-qPCR analysis to detect *PGR-AB* (A) and *PGR-B* (B) expression. *ACT-B* was used as a reference, constitutively expressed gene. Values shown in the figures are means and SEM obtained from 3 independent cultures. **a** P≤0.05 vs. vehicle, E2, MPA, E2+MPA; **b** vs. cAMP; **c** vs. E2+cAMP (ANOVA, Tukey).

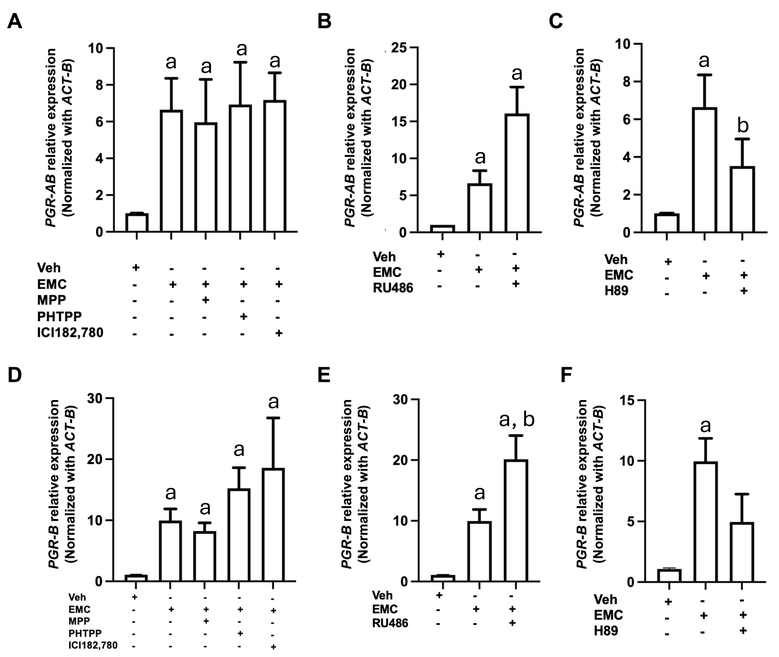


Figure 3. Effect of ERs and PR antagonists and PKA inhibitors in the regulation of *PGR-AB and PGR-B* isoforms expression during decidual stimulus in T-HESCs. T-HESCs were preincubated with an ER antagonist ICI182,780, selective ERα antagonist MPP, selective ERβ antagonist PTHPP (A, D), PR antagonist RU486 (B, E), and PKA inhibitor H89 (C, F), followed by incubation E2+ MPA+ cAMP (EMC) or ethanol+ DMSO (vehicle) for 24 h. The RNA was subjected to RT-qPCR analysis to detect *PGR-AB* (A, B, C) and *PGR-B* (D, E, F) expression. *ACT-B* was used as a reference, constitutively expressed gene. Values shown in the figures are means and SEM obtained from 3 independent cultures. **a** P≤0.05 vs. vehicle; **b** vs. EMC (ANOVA, Tukey).

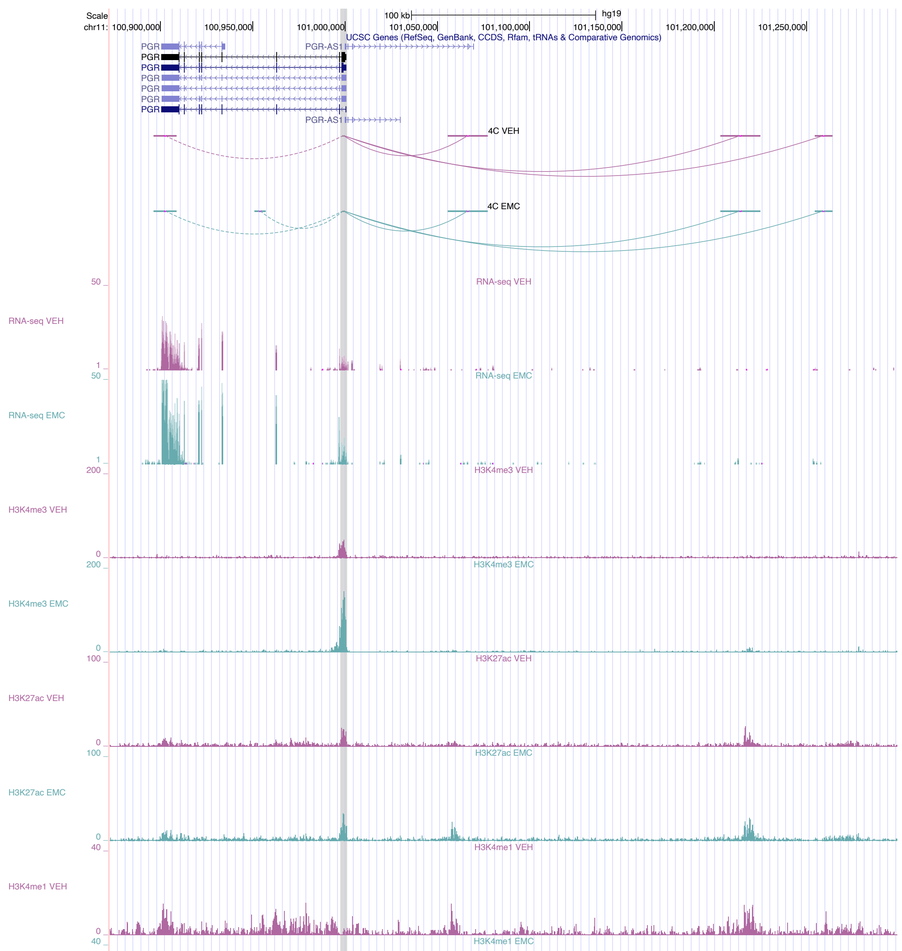


Figure 4. Schematic representation of RNA-seq, ChIP-seq, and 4-C contact maps centered on the progesterone receptor gene (*PGR*) in T-HESC cells in *in vitro* decidualization. T-HESCs were treated with either vehicle or E2, MPA, and cAMP (EMC) for 24 hours. The genomic regions interacting with the *PGR* promoter (anchor) were identified by 4C-seq using Csp6I and DpnII as restriction enzymes. Arcs in the track indicate significant interactions between the *PGR* gene promoter and putative distal regulatory elements identified through 4C-seq. Each histone modification and RNA-seq track displays read counts per base pair for each experiment. Representative replicate result from three replicates are shown for cells treated with vehicle (purple) and EMC (green).

Discussion and conclusion:

Our findings demonstrate that during *ss*decidualization of T-HESCs with EMC, cAMP induces the expression of *PGR-AB* and *PGR-AB* (Fig. 1-2) by activating the PKA signaling pathway (Fig. 3), while MPA downregulates their expression through the PR (Fig. 2-3). This induction is associated with increased enrichment of H3K4me3 and H3K27ac in the promoter region of *PGR* after 24 h of EMC treatment (Fig. 4).

We also identified four potential distal regulatory regions that interact with the *PGR* promoter, enriched with H3K27ac and H3K4me1 when T-HESCs were treated with the vehicle (Fig. 4). Interestingly, a new interaction was observed when T-HESCs were treated with EMC; however, the corresponding distal region did not show enrichment in H3K4me1 and H3K27ac.

Our results suggest pre-existing interactions between distal regulatory regions and the *PGR* promoter, maintaining the basal expression of this gene. Additionally, treatment with EMC could enhance the recruitment of basal transcription machinery probably via cAMP-PKA signaling pathway, leading to increased expression and elevated levels of H3K4me3 and H3K27ac in the promoter region (Fig. 4).

References:

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Acknowledgements:

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