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— From Innovation to Impact —

Session PL01 - President's New Investigator Plenary

Add To Itinerary

O-002 - Novel Non-Classic Progesterone (P4) Receptor PGRMC1 Interactions and Functionality Reveal a Key Role During the Human Decidualization Process.

March 14, 2019, 9:15 AM - 9:30 AM | Ampi Bleu

Categories
+11.2 - Basic Reproductive Biology: Implantation, Endometrium

Keywords
Progesterone receptor,Decidualization,PGRMC1

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Abstract

Introduction: Progesterone (P4) Receptor Membrane Component 1 (PGRMC1) mediates the antiapoptotic and antimitotic actions of P4 in human granulosa cells. However, its function in the endometrium remains unknown. We previously demonstrated that PGRMC1 is down-regulated in receptive endometria and its overexpression inhibits decidualization. Here, we investigated interactions of PGRMC1 with other proteins and the effect of a PGRMC1 inhibitor (AG205) during decidualization.

Methods: PGRMC1 protein interactions were identified in non-decidualized (ndESC) and decidualized (dESC) endometrial stromal cells through a pulldown assay during a long or short decidualization protocol (P4/E2;8 days or cAMP/MPA;4 days). PGRMC1 sequence was cloned in a pGEX-6P1 vector to express a GST-PGRMC1 fusion protein. Proteins extracted from ndESC and dESC (n=3) were incubated with GST-PGRMC1 or control GST proteins and subsequently identified by mass spectrometry (MS). To better understand the role of PGRMC1 in decidualization, an impermeable P4 (P4-BSA, 1uM) that activates only membrane receptors and the inhibitor AG205 (50 uM)(n=7) were used to block its action. Decidualization was evaluated by analyzing prolactin (PRL) secretion (ELISA) and cytoskeleton morphology (F-actin staining). Global gene expression following AG205 and P4-BSA treatment during a long protocol of decidualization was analyzed by microarray and validated by qPCR (n=4).

Results: Pulldown and MS analysis identified 22 and 25 new significant PGRMC1-interacting proteins in ndESC and dESC, compared to controls ($p<0.01$). Interaction network analysis categorized these proteins mainly into mitochondria and lysosome cellular components, both related to transport activity. Monoamine oxidase B (MAOB) was identified in dESC of both decidualization protocols. The PGRMC1-MAOB interaction was confirmed by immunofluorescence and co-immunoprecipitation in dESC. PRL secretion significantly decreased in the presence of P4-BSA/E2 compared to dESC by P4/E2 ($p<0.05$). Furthermore, PRL levels were significantly increased in the presence of AG205+P4-BSA/E2 compared to ndESC and dESC only with P4-BSA/E2 ($p<0.05$). Finally, microarray analysis showed that AG205 was associated with 91 up- and 147 down-regulated genes compared to ndESC (FDR<0.05). Moreover, biological processes associated with cholesterol/sterol biosynthesis and vesicle-mediated transport were up-regulated.

Conclusion: Novel PGRMC1 protein interactions discovered in ESC and PGRMC1 functional analysis suggest that this protein is implicated in the deep remodeling of ESC during the decidualization process, interacting mainly with proteins involved in intracellular transport and mitochondrial activity.