GRANULOSA CELL APOPTOSIS

TRANSCRIPTIONAL REGULATION BY THE NUCLEAR PROGESTERONE RECEPTOR

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Göteborgs universitet kommer att försvaras offentligt i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, torsdagen den 12 mars 2009, kl. 09.00

av

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Avhandlingen baseras på följande arbeten:

I **Progesterone-receptor antagonists and statins decrease de novo cholesterol synthesis and increase apoptosis in rat and human periovulatory granulosa cells in vitro.**
Rung E, Friberg PA, Shao R, Larsson DGJ, Nielsen E, Svensson PA, Carlsson B, Carlsson LM, Billig H.
Biol Reprod 2005; 72: 538-545.

II **Apoptotic effects of a progesterone receptor antagonist on rat granulosa cells are not mediated via reduced protein isoprenylation.**
Friberg PA, Larsson DGJ, Rung E, Billig H.
Mol Reprod Dev 2007; 74: 1317-1326.

III **Dominant role of nuclear progesterone receptor in the control of rat periovulatory granulosa cell apoptosis.**
Friberg PA, Larsson DGJ, Billig H.
Biol Reprod 2009; In Press as DOI:10.1095/biolreprod.108.073932.

IV **Nuclear progesterone receptor in rat periovulatory granulosa cells: genome-wide transcriptional regulation by Org 31710 in vitro.**
Friberg PA, Larsson DGJ, Billig H.
Submitted.

UNIVERSITY OF GOTHENBURG

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TRANSCRIPTIONAL REGULATION BY THE NUCLEAR PROGESTERONE RECEPTOR

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ABSTRACT

Ovarian follicle atresia caused by granulosa cell apoptosis is a central process in normal female physiology. Progesterone has been reported to be a survival factor in granulosa cells at several developmental stages. This thesis focuses on the local functions of progesterone relating to the control of granulosa cell apoptosis during the periovulatory interval. The well-characterized gonadotropin-primed immature rat model was used to generate periovulatory granulosa cells, which were subsequently subjected to serum-free cell culture. The effects mediated by the nuclear progesterone receptor were investigated using two progesterone receptor antagonists, RU 486 (mifepristone) and Org 31710. The transcriptional regulation mediated by the nuclear progesterone receptor was investigated using the Affymetrix microarray technique. Decreased de novo synthesis of cholesterol was found to be one of the major effects of high concentrations of Org 31710. Recent studies have demonstrated that inhibition of cholesterol synthesis results in substrate limitation for post-translational isoprenylation, which has interesting implications for the cellular control of apoptosis. We found that cholesterol synthesis and protein isoprenylation are important factors maintaining granulosa cell survival; however, decreased protein isoprenylation cannot explain the induction of apoptosis by progesterone receptor antagonists. In addition to transcriptional regulation, progesterone also initiates rapid cellular responses that have been suggested to regulate granulosa cell apoptosis. We have demonstrated that Org 31710, which acted on the nuclear progesterone receptor, specifically and reversibly induced apoptosis of periovulatory granulosa cells in vitro. We found no support for any contributing non-genomic signaling of progesterone. Furthermore, we could not corroborate previous reports suggesting rapid effects of progesterone in immature rat follicles. Expanded microarray studies focused on early and late transcriptional effects of low doses of Org 31710. Gene ontology analysis was used to select biologically relevant functional groups for further analyses, including genes involved in apoptosis, reproductive processes, cell adhesion, cell cycle regulation, transcriptional control and angiogenesis. In conclusion, we found that progesterone is a central, survival-promoting regulatory factor that acts via the nuclear progesterone receptor during the periovulatory interval. The identification of novel gene targets of progesterone expands our knowledge of the events that occur in granulosa cells during ovulation and luteinization.

Keywords: ovary, granulosa cells, ovulation, luteinization, progesterone, apoptosis, cholesterol synthesis, isoprenylation

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