

## **Antiproliferative Proteins in Ovulation in Rat Ovary**

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The LH surge triggers ovulation by activating cascade of genes. We investigated ovulation-associated genes using DNA microarray analysis and differentially expressed gene (DEG) kit. Interestingly, factors important for the transition from proliferation to differentiation were stimulated by LH. These factors include genes regulated by the activation of PKC zeta, interferon alpha, B-cell translocation gene (Btg) and cyclin G. Indeed, FACS analysis demonstrated that an increase in G<sub>0</sub>/G<sub>1</sub> phase versus a decrease in S phase was detected within 6-8 hr after LH treatment, indicating the transition of granulosa cells. In vitro kinase assay revealed the activation of PKC zeta within 20 minutes after LH treatment in preovulatory follicles. Genes regulated by PKC zeta pathway were then identified using DEG kit. Thirteen genes including testin, aminolevulinic acid synthase 1 (Alas1), retrovirus SC1 were stimulated by PKC zeta activation. Interestingly, many genes regulated by PKC zeta pathway were involved in mitochondrial function. Btg2 gene expression was induced by LH within 1 hr in granulosa cells of preovulatory follicles. Furthermore, activation of MAP kinase pathway was responsible for LH-induced Btg2 expression. Yeast two-hybrid screening of follicle library with Btg2 as a bait resulted in the detection of several candidate genes for interaction including adenine nucleotide translocator 2 (ANT2), 3 alpha hydroxysteroid dehydrogenase, and strawberry notch homology 1. GST pull-down assay revealed a direct interaction of Btg2 with ANT2. Furthermore, mutant analysis identified the specific domain of Btg2 important for interaction. Interaction of Btg2 with ANT2 modulated ANT2 function such as mitochondria depolarization, ATP production and cell proliferation. Our findings demonstrate that proteins controlling mitochondria function play role in the process of transition from proliferation to differentiation which is required for LH-induced ovulation.