P-13 Interaction of B-cell Translocation Gene 2 with Adenine Nucleotide Translocator 2 in Granulosa Cells of Preovulatory Follicles of Rat Ovary

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Objectives: The present study was to examine the gonadotropin regulation and mechanism of action of BTG2 during the ovulation in immature rats.

Methods: Ovaries were also collected from immature (26-day-old) rats at various times after treatment with 10 IU PMSG for Northern blot, Western blot, and in situ hybridization analyses. Granulosa cells of preovulatory follicles were also collected by the method of follicular puncture using 23-gauge needles at different time intervals. Coimmunoprecipitation was utilized to confirm interaction of BTG2 with ANT2. To confirm colocalization of BTG2 and ANT2, subcellular fractionation was performed. GST pull down assay was performed to elucidate BTG2 domain interacting with ANT2 on modulating the function of ANT2 by generating BTG2 deletion mutants. ATP level in the preovulatory granulosa cells was determined by bioluminescence using a spectrofluorimeter with the ATP Bioluminescence HS II assay kit. Change of mitochondrial membrane potential difference was tested by FACS analysis with JC-1 stain of the living cells.

Results: Btg2 expression was transiently stimulated by LH/hCG in preovulatory granulosa cells. Interestingly, GST pull-down and coimmunoprec-ipitation assay demonstrated that Btg2 physically interacted with adenine nucleotide translocator (ANT) 2, a mitochondrial transmembrane protein. BTG2 and ANT2 proteins were colocalized in the mitochondrial fraction in transfected 293T cells. Deletion analysis of BTG2 demonstrated that the N-terminal end of Btg2 was responsible for interacting with ANT2 protein. Lastly, BTG2 modulated ANT2 functions on mitochondrial depolarization and ATP production.

Conclusion: In conclusion, Btg2 stimulated by LH/hCG interacts with ANT2, resulting in the modulation of ANT2 function and thus may exert its anti-proliferative role during the ovulatory process.

P-14 Egr1, a Critical Factor for Ovulation in the Mouse Ovary, is Transiently Induced by LH Signaling Pathway

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Objectives: The Egr family of zinc finger transcription factors consisting of 4 members regulates critical genetic programs involved in cellular growth, differentiation, and function. They are co-expressed in many different tissues, suggesting that they may have some redundant functions. Egr1(−/−) female mice showed infertility due to anovulation resulting from luteinizing hormone β subunit (LHβ) deficiency. While it is clear that Egr1 regulates transcription of LHβ in the pituitary gland, the roles of Egr1 in the ovary still remain unexplored. Thus, we have examined temporal expression profiles of Egr family and their cofactors (Nab1 and Nab2) in the ovary during ovulation process and whether Egr1 expressed in the ovary