

THE EFFECT OF EXTRACELLULAR ATP INDUCED MAPK SIGNALING IN HUMAN ENDOMETRIAL STROMAL CELLS AND CROSS TALK BETWEEN ATP AND STEROID HORMONES

Shu-Ju Chang¹, Chii-Ruey Tzeng^{2,3}, Wei-Min Liu^{2,3}, Chen-Jei Tai^{1,3}

¹ Department of Traditional Chinese Medicine, Taipei Medical University Hospital (Taiwan)

² Department of Obstetrics and Gynecology, Taipei Medical University Hospital (Taiwan)

³ Department of Obstetrics and Gynecology, College of Medicine, Taipei Medical University (Taiwan)

Purpose: Our previous data demonstrated that ATP activated mitogen-activated protein kinases (MAPKs) through the P2Y2 receptor/PLC/PKC/ERK signaling pathway and inhibited cell proliferation in human endometrial stromal cells (hESCs). However, little is known about the cross talk between ATP and steroid hormones.

Materials and Methods: Samples of proliferative phase endometrium were obtained from 45 cycling women (ages 27-48 years) undergoing hysterectomy that had no history of malignancy. Patient informed consent was obtained from the patients prior to tissue collection. The use of human endometrium was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects of Taipei Medical University Hospital. The endometrium was digested with 0.1% collagenase and 0.1% hyaluronidase, and hESCs were cultured in 10% FBS-supplemented DMEM. To examine the ATP, hormone, and ERK1/2 relationship, hESCs were treated with 10 μ M ATP, 100nM GnRH, E2, P4, 10 IU/ml HCG, and alcohol for 10 min. To examine the dose-response relationship, hESCs were treated with increasing concentrations of estrogen, or progesterone (0, 1 nM, 10 nM, 100 nM, 1 μ M) for 10 min. For time-course experiments, hESCs were treated with 100 nM estrogen, or progesterone for 0, 5, 10, 20, 30 min or 0, 1, 5, 10, 20 min. Western blot analysis was performed using antibodies against the phosphorylated forms of ERK1 and ERK2. ERKs activity was expressed as a relative ratio of basal levels. Independent replicates of experiments in this study were performed with cells from 3 different patients. Data were represented as means $\pm$ standard errors (SE). Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparison tests. Differences were considered significant at $p < 0.05$.

Results: We reported previously the effects of extracellular ATP on MAPKs in hESCs. A significant effect of ATP on ERK1/2 activation was observed at 10 μ M, and the maximal effect was noted at the 10-min time-point. Western blot analysis, which detected the total and phosphorylated forms of ERK1/ERK2, demonstrated that exogenous estrogen or progesterone activated ERK1/2 in a dose- and time-dependent manner. In time-course experiments, estrogen was capable of rapidly inducing ERK1/2 activity. A significant effect was seen within 10 min after treatment, and the activation of ERK1/2 was sustained for at least 20 min. As shown in the study, the effect of estrogen reached maximum at 30 min. In addition, progesterone was capable of inducing ERK1/2 activity promptly. A significant effect was seen within 5 min after treatment, and the effect of progesterone reached maximum at 10 min.

Conclusion: This study demonstrated that ATP, estrogen, and progesterone are able to activate ERK1/2 in hESCs, evoking our curiosity to examine the cross-talk between ATP and estrogen, or progesterone in hESCs.