P17 ATP induces nuclear translocation of ERK1/2 in human endometrial stromal cells

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Objectives: With the stimulation of many types of cell surface receptors, mitogen-activated protein kinases (MAPKs) are activated. However, little is known about the specific role of ATP in the subsequent MAPK-induced signaling cascade in human endometrial stromal cells (hESCs). The present study was designed to examine the effect of ATP on MAPK activity and the translocation of activated ERK1/2 in hESCs.

Materials and Methods: Samples of endometrium were obtained from women undergoing hysterectomy with no history of malignancy. The endometrium was digested with 0.1% collagenase and 0.1% hyaluronidase, and hESCs were cultured in 10% FBS-supplemented DMEM. To measure MAP kinase activity, a nonradioactive method was used (p44/42 MAP Kinase Assay Kit). The nuclear translocation of activated ERKs was examined using a confocal laser scanning microscopy. Human ESCs were grown onto glass coverslips and incubated for 3 d at 37℃ in humidified air with 5% CO_2. Cells were treated with 10 μM ATP for 10 min in the absence or presence of PD98059 (pretreated for 30 min before ATP exposure). Goat anti-mouse IgG (H+L) or goat anti-rabbit IgG (H+L) and Cy™2-conjugated * streptavidin or Cy™3-conjugated * streptavidin antibodies were used to detect the distribution of total and phosphorylated ERKs.

Results: MAP kinase activity assay showed that ATP significantly increased MAPK activity by 250% of the control level. Immunofluorescent staining revealed that phosphorylated ERKs were translocated from cytoplasm into nucleus subsequent to 10μ M ATP treatment.

Conclusion: To our knowledge, this is the first demonstration of the ATP-induced nuclear translocation of ERK1/2 in the hESCs. These results suggest that the ERK1/2 signaling pathway plays a role in mediating ATP actions in hESCs, implying that ATP might play an important role in human reproductive system.

Key words: ATP, ERK1/2, human endometrial stromal cell

P18 Extracellular ATP induces mitogen-activated protein kinases (MAPK) signaling pathway in human endometrial stromal cells

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Objectives: Extracellular ATP has been shown to activate the phospholipase C (PLC) / protein kinase C (PKC) pathway. However, little is known about the downstream signaling events. The present study was designed to examine the effect of ATP on activation of mitogen-activated protein kinase (MAPK) signaling pathway in human endometrial stromal cells (hESCs).

Materials and Methods: Samples of endometrium were obtained from women undergoing hysterectomy with no history of malignancy. The endometrium was digested with 0.1% collagenase and 0.1% hyaluronidase, and hESCs were cultured in 10% FBS-supplemented DMEM. To examine the dose-response, hESCs were treated with increasing concentrations of ATP (100 nM, 1 μM, 10 μM or 100 μM) for 5 min. For time-course experiments, hESCs were treated with 10 μM ATP for 1, 5, 10 or 20 min. Western blot analysis was performed using antibodies against the phosphorylated forms of ERK1 and ERK2. To explore the ATP-activated intracellular signaling pathway, hESCs were treated with suramin (a P2-purinoceptor antagonist), neomycin (a PLC inhibitor), staurosporin (a PKC inhibitor) or PD 98059 (a MEK inhibitor).

Results: Western blot analysis, which detected the total and phosphorylated forms of ERK1/ERK2, demonstrated that exogenous ATP activated MAPK in a dose-and time-dependent manner. Treatment of the cells with suramin, neomycin, staurosporin, PD 98059 significantly attenuated the ATP-induced activation of MAPK.

Conclusion: ATP, after binding to a P2 purinoceptor, activated MAPKs through PLC/PKC signaling pathway in hESCs. To our knowledge, the present study is the first one demonstrating the effect of ATP on hESCs, implying that ATP might play an important role in human reproductive system.

Key words: ATP, MAPKs, human endometrial stromal cell