ATP 在人類子宮內膜基質細胞中所扮演角色之探討

The Role of Adenosine Triphosphate in Human Endometrial Stromal Cells

中文摘要

細胞外的腺嘌呤核甘三磷酸 (ATP) 主要與細胞膜上的 P2Y 嘌呤受體 (purinoceptor) 結合，進而引發一連串的細胞內訊息傳遞路徑; 據研究顯示，可對於多種不同的細胞類型引起各種不同的生物反應。研究證實在各種不同的生理系統中，ATP 主要活化 G-protein, phospholipase C (PLC), diacylglycerol (DAG), protein kinase C (PKC) 之細胞內訊息傳遞路徑。然而對於它在人類子宮內膜基質細胞的作用機制並不清楚。

本研究的設計主要分別從 mRNA 及 protein level 檢測人類子宮內膜基質細胞 P2Y2 嘌呤受體存在之情形。進而探討在人類子宮內膜基質細胞，細胞外的 ATP 活化 mitogen-activated protein kinase (MAPK) 的訊息傳遞路徑及對早期即時表現(immediate early genes)基因和細胞生存能力(cell viability)的影響。研究的證實運用西方墨點分析法 (Western blot analysis)、基因組膜片分析法、細胞生存能力以 MTT 分析法。

分別從 mRNA 及 protein level 證實人類子宮內膜基質細胞存在有 P2Y2 嘌呤受體。西方墨點分析法 (Western blot analysis)；以 ERK1/ERK2 (p42mapk 和 p44mapk) 個別磷酸化形式的單株抗體來做偵測，證實 ATP 與 UTP 活化 ERK1/2 會隨濃度及時間的不同而產生變化。細胞以 suramin (P2-嘌呤受體拮抗劑)，neomycin (PLC 抑制劑)，staurosporin (PKC 抑制劑) or PD98059 (MEK,MAPK/ERK kinase, 抑制劑) 處理後，其因 ATP 而活化之 ERK1/2 的表現很明顯被弱化。以細胞免疫螢光染色法和 Western blot analysis，證實 ATP 所引發活化之 ERK1/2 將訊息由細胞質轉移至細胞核時，活化之 ERK1/2 本身所產生之移位作用。在 10 μM ATP 處理前先加入 PD98059 抑制劑則活化之 ERK1/2 本身所產生之移位作用的表現很明顯被弱化。爲進一步探討 ATP 在細胞核內所引起之基因的改變及生長的改變；細胞以 10 μM ATP 處理 24 小時後萃取其 mRNA，使用經由 mitogen 的訊息傳遞鏈所引起 matrix metalloproteinases (MMPs)表現之 96 個基因組膜片，相較於未經 10 μM ATP 處理之對照組，證實 MMP-2, -3, -10, -24 的基因表現量有明顯增加；在 10 μM ATP 處理前先加入 PD98059 抑制劑則 MMP-2, -3, -10, -24 的基因表現量很明顯被弱化。更進一步以 RT-PCR 半定量法證實不同劑量 ATP 對於 MMP-2, -3, -10, -24 的基因表現量之影響。細胞以 10 μM ATP 處理 30 分鐘後萃取其 mRNA，使用經由 mitogen 的訊息傳遞鏈所引起早期即時表現之 23 個基因組膜片，相較於未經 10 μM ATP 處理之對照組，證實 early growth response 1 的基因表現量有明顯增加；在 10 μM ATP 處理前先加入 PD98059

抑制劑則 early growth response 1 的基因表現量很明顯被弱化。以 MTT assay 分析 cell viability，證實 10 μM ATP 及 100 μM ATP 藉由引發 ERK1/2 訊息傳遞路徑抑制 cell viability。
在人類子宮內膜基質細胞之研究中所得到的結論，在細胞核內基因的影響方面--細胞外的 ATP 經由 P2Y2/ PLC/PKC/ERK1/2 的訊息傳遞路徑，使得 MMP-2, -3, -10, -24 的基因表現量和 early growth response 1 的基因表現量有明顯增加。在細胞生長的影響方面--細胞外的 ATP 經由 P2Y2/ PLC/PKC/ERK1/2 的訊息傳遞路徑抑制 cell viability。

英文摘要

ATP is an extracellular signaling molecule that activates specific G protein-coupled P2Y receptors in most cell types to mediate diversely biological effects. ATP has been shown to activate the phospholipase C (PLC)/ diacylglycerol/ protein kinase C (PKC) pathway in various systems. However, little is known about the signaling events in human endometrial stromal cells (hESCs).
The objective of this study was to examine the presence of the P2Y2 receptor and the effects of exogenous ATP on the intracellular mitogen-activated protein kinases (MAPKs) signaling pathway, immediate early genes expression, and cell viability in hESCs. Western blot analysis, gene array analysis, and MTT assay for cell viability were performed.
The current study demonstrated the existence of the P2Y2 purinergic receptor at the mRNA and protein level in hESCs. UTP and ATP activated ERK1/2 in a dose- and time-dependent manner. Suramin (a P2-purinoceptor antagonist), neomycin (a PLC inhibitor), staurosporin (a PKC inhibitor), and PD98059 (a MEK inhibitor) significantly attenuated the ATP-induced activation of ERK1/2. Confocal microscopy and Western blot analysis showed an evident nuclear translocation of phosphorylated ERKs after 10 μM ATP treatment, but this effect was blocked by PD98059. To study the gene(s) induced by exogenous ATP, mRNA was extracted from hESCs in the presence or absence of 10 μM ATP. The gene array for 96 genes associated with members of human matrix metalloproteinases (MMPs) and adhesion molecules revealed that the expression of MMP-2, -3, -10, and -24 genes was increased and the effect was attenuated by PD98059. Furthermore, the effects of ATP on the expression of MMP genes were confirmed by semiquantitative RT-PCR. The gene array for 23 genes associated with members of the mitogenic pathway cascade and immediate early genes revealed that the expression of early growth response 1 was increased. In addition, MTT assay revealed an inhibition effect of ATP on cell viability.
ATP activated ERK1/2 through the P2Y2 purinoceptor/PLC/PKC/ERK signaling pathway and induced translocation of ERK1/2 into the nucleus. Further, ATP induced

the expression of early growth response 1 and inhibited cell viability in hESCs. To our knowledge, this is the first demonstration of the ATP-induced nuclear translocation of phospho-ERK1/2 that mediates MMPs gene expression in human endometrial cells. These results support the notion that the ERK1/2 signaling pathway is involved in mediating ATP actions in the human reproductive system.