p44/42 MAPK及NF-κB 在Phorbol-12-Myristate-13-Acetate引發人類肺臟上皮細胞環氧酵素-2 表現的訊息傳遞路徑之角色

The Role of p44/42 MAPK and NF-κB on Phorbol-12-Myristate-13-Acetate-Induced Cyclooxygenase-2 Expression in Human Pulmonary Epithelial Cells (A549)

中文摘要

本論文主要探討 phorbol-12-myristate-13-acetate (PMA) 引發人類肺臟上皮細胞（A549）之 PGE2 釋放、COX 活性增加及 COX-2 表現之訊息傳遞路徑以及 PKC 同功酵素 (isoforms) 在此路徑所扮演的角色。PMA 以劑量及時間相關的方式刺激 PGE2 釋放、COX 活性增加及 COX-2 表現。而蛋白轉錄抑制劑 actinomycin D 和蛋白轉譯抑制劑 cycloheximide 可抑制 PMA 引發 PGE2 釋放、COX 活性增加及 COX-2 表現。PKC 抑制劑（Ro 31-8220, Go 6976）或以 PMA (1 μM) 長時間 (24 hr) 處理也會減少 PMA 所引發之 PGE2 釋放、COX 活性增加及 COX-2 表現，然而 Ras 活化的抑制劑 FPT inhibitor II 則沒有任何影響。先前的研究報告已指出，在 A549 細胞中存在有 PKC-α,-γ,-ι,-λ,-μ 及 -ζ 六種 isoforms。以 PMA (1 μM) 刺激 A549 細胞後，發現只有 PKC-α 及 PKC-γ 兩種 isoforms 會從細胞質轉位到細胞核中，其它 isoforms 則否。而以 PMA (1 μM) 長時間 (24 hr) 處理 A549 細胞會造成 PKC-α 及-γ 的 down-regulation，意味著 PKC-α 及-γ 包含於 PMA 引發 COX-2 表現的訊號傳遞路徑當中。

MEK 抑制劑 PD 98059 可抑制 PMA 所引發之 PGE2 釋放、COX 活性增加及 COX-2 表現，然而 p38MAPK 抑制劑 SB 203580 對 PMA 所引發的反應則沒有任何影響。以 PMA 刺激 A549 細胞可導致 p44/42 MAPK 的活化，當加入抑制劑 Ro 31-8220、PD 98059、genistein、SB 203580 或長時間 (24 hr) 之 PMA 處理，發現 Ro 31-8220、PD 98059 及長時間 PMA 的處理皆會抑制 p44/42 MAPK 的活化，genistein 及 SB 203580 則沒有抑制作用。 NF-κB 抑制劑 pyrrolidine dithiocarbamate (PDTC) 及 Iκ-Bα protease 抑制劑 TPCK 皆可抑制 PMA 所引發之 PGE2 釋放、COX 活性增加及 COX-2 表現。以 PMA 刺激 A549 細胞可引發 p65 NF-κB 從細胞質轉位到細胞核，以及細胞質中 Iκ-Bα 的分解。當預先以 Ro 31-8220、PD 98059、 SB 203580 及 PDTC 處理 A549 細胞，發現 Ro 31-8220、PD 98059 及 PDTC 皆可抑制 p65 NF-κB 的轉位，只有 SB 203580 沒有抑制作用。由以上結果說明，在 A549 細胞中，PMA 是藉由先活化 PKC 之後再使 p44/42 MAPK 活化，接著導致 NF-κB 的活化，最後才導致 COX-2 表現及 PGE2 釋放。而存在於 A549 細胞中的 PKC isoforms，只有 PKC-α 及 PKC-γ 是包含在 PMA 引發 COX-2 表現及 PGE2 釋放的反應當中。

英文摘要

We examined the role of PKC isoforms and signaling pathway involved in phorbol-12-myristate-13 acetate (PMA)-induced prostaglandin E2 (PGE2) release, the increase of cyclooxygenase (COX) activity and COX-2 expression in human pulmonary epithelial cell line
(A549). PMA caused a concentration- and time-dependent increase in PGE2 formation, the increase of COX activity and COX-2 expression. Actinomycin D and cycloheximide inhibited PMA-induced PGE2 release, the increase of COX activity, and COX-2 expression. The PKC inhibitors (Go 6976 and Ro 31-8220) or long-term (24 hr) PMA treatment attenuated PMA-induced PGE2 release, the increase of COX activity and COX-2 expression, while FPT inhibitor II (Ras farnesyl protein transferase inhibitor) had no effect on PMA-induced responses. The tyrosine kinase inhibitor, genistein, attenuated PMA-induced PGE2 release, but not PMA-induced the increase of COX activity and COX-2 expression. In our previously studies have demonstrated that PKC-α, -γ, -ι, -λ, -μand -ζwere detected in A549 cells. Treatment of A549 cells with PMA (1 μM) caused the translocation of PKC-α and -γbut not other isoforms from cytosol to the membrane fraction. Long-term treatment of PMA (1μM) resulted in complete down-regulation of PKC-α and -γ, indicating the activation of PKC-α and -γis involved in PMA-mediated responses. The MEK inhibitor (PD 98059) attenuated PMA-induced PGE2 release, the increase of COX activity and COX-2 expression, while p38 mitogen-activated protein kinase inhibitor, SB203580, had no effect. Treatmemt of A549 cells with PMA caused p44/42 MAPK activation ; the activation was inhibited by Ro 31-8220, PD 98059 or long-term PMA treatment, but not genistein or SB 203580. The NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC) and the Iκ-B protease inhibitor, l-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK), prevented PMA-induced PGE2 release, the increase of COX activity, and COX-2 expression. PMA also caused the translocation of p65 NF-κB from cytosol to the nucleus as well as the degradation of Iκ-Bαin cytosol. Furthermore, the PMA-induced p65 NF-κB activation was inhibited by Ro 31-8220, PD 98059 or PDTC, but not SB 203580.These results indicate that PMA might activate PKC to elicite p44/42 MAPK activation, which in turn initiates NF-κB activation, and finally induces COX-2 expression and PGE2 release in A549 cells. Of the PKC isoforms present in A549 cells, only PKC-α and -γactivation are involved in regulating the PMA-induced responses.