MMP-9 與 COX-2 在 TPA 誘導之人類神經膠質瘤轉移之角色探討

Roles of MMP-9 and COX-2 in TPA-induced metastasisi of human glioblastoma cells

中文摘要

神經膠質瘤(Glioblastoma)爲腦癌中最惡性且轉移和侵犯能力最強的一種原發性腦癌。爲了要瞭解神經膠質瘤細胞如何侵犯和轉移的機制，本實驗利用人類神經膠質瘤細胞株(U87MG)探討12-o-tetradecanoylphorbol 13-acetate(TPA)所誘導產生的基質金屬蛋白酶(Matrix metalloproteinases-9, MMP-9)表現和環氧酶(Cyclooxygenase, COX)活化在癌細胞轉移和侵犯的機制中所扮演的角色。利用 Spheroid assay 和 wound migration assay 和 In vitro invasion assay 三種體外實驗，探討 TPA 所誘導的神經膠質瘤細胞的轉移和侵犯的生理活性現象。TPA 本身是很強的細胞癌化促進劑，在動物皮膚試驗及細胞實驗中均被證實。TPA 之所以能夠促進細胞癌化是經由活化細胞內的訊息傳導路徑。在 U87MG 細胞中，TPA 所誘導之 MMP-9 酵素活性和 COX-2 蛋白的表現是相呼應的。若使用 COX-2 抑制劑(NS398)則可抑制 MMP-9 的酵素活性並且顯著地降低 TPA 所誘導之癌細胞轉移和侵犯的現象。在加入 TPA 的 U87MG 細胞中可以偵測到 ERKs 透過蛋白質的磷酸化而活化，並且如果將細胞培養在加入 ERKs 抑制劑 PD98059 中，可以明顯地降低 TPA 所誘導的 MMP-9 和 COX-2 蛋白表現進而抑制細胞的轉移和侵犯。除此之外，TPA 也可刺激 U87MG 細胞中 protein kinase C-?? (PKC??)活性的增加。加入了 PKC 的抑制劑(GF109203X, GF 和 Go6976, Go)可以明顯的抑制由 TPA 所誘導的 ERKs 磷酸化和 COX-2 蛋白及 MMP-9 酵素的活性表現。同時，我們也發現活性氧物質 Reactive oxygen species(ROS)的活化也包含在此訊息傳導的路徑中。在加入了 ROS 抑制劑 Superoxide dismutase(SOD),Tempol(TEM)和 Diphenylene iodonium(DPI)後，可抑制由 TPA 所誘導產生的 ROS 和細胞中的訊息傳遞。本篇實驗證實了 MMP-9 酵素活性和 COX-2 蛋白的活化在 TPA 所誘導的神經膠質瘤細胞轉移和侵犯中扮演了相當重要的角色，而細胞內的激酶與 ROS 的活化、COX-2 基因的表現也參與其中。

英文摘要

Astrocytic glioma is a type of malignant brain tumor and the mortality of patients with astrocytic glioma remains high in the world. Therefore, investigating transformation mechanism of glioma, and developing effective agents to block its transformation are important issues for glioma treatment. In the present study, tumor promoter 12-o-tetradecanoylphorbol 13-acetate (TPA) addition induces migration, invasion, and transformation in U87MG glioblastoma cells by spheroid formation and wound healing assay and transwell invasion assay accompanied by stimulating MMP-9 enzyme activity. A significant increase in COX-2 protein and mRNA level was observed in TPA-treated U87MG glioma cells, and adding chemical COX inhibitor NS398 reduces the TPA-induced MMP-9 activation and transformation in U87MG cells. Suppression of ERKs, but not JNKs, by chemical inhibitors TPA-induced transformation reduced MMP-9 enzyme activity, and COX-2 protein in

U87MG cells. Addition of PKC inhibitors GF109203X(GF) and Go6976(Go) significantly attenuates TPA-induced transformation in according with suppressing ERKs protein phosphorylation, and membrane translocation of PKC?? protein. Suppression of TPA-induced transformation events by reactive oxygen species scavengers Superoxide dismutase(SOD),Tempol(TEM) and Diphenylene iodonium(DPI) were identified in the present model. These data indicate that activation of PKC-ERKs via ROS production is involved in the transformation and MMP-9 activation of glioma in response to TPA, and COX-2-dependency are identified.