Thrombin 刺激肺部上皮細胞誘導介白素-8 表現經由

Gβγ2 依賴 Rac 及 PI3K/Akt 的活化

Thrombin Induces Interleukin-8 Expression Through Gβγ2-Dependent Rac and PI3K/Akt Activation in Human Pulmonary Epithelial Cells

中文摘要

Thrombin 為一個多功能的絲胺酸蛋白?，可從受傷的血管釋放來，為參與凝血反應重要的凝血因子。此外，它也可?生多方面的胞生理反應，其中包括調節發炎反應。根據先前研究指出，在呼吸道上皮細胞中，thrombin 可誘導前發炎物質 IL-8 的產生，然而其中作用機制仍不清楚。本篇論文主要探討主題在 thrombin 刺激肺部上皮細胞?生 IL-8 表現是否經由 Gβγ2、Rac 及 PI3K/Akt 之訊息傳導而來。

在 A549 肺部上皮細胞中，thrombin 誘導 IL-8 的釋放呈現濃度相關反應曲線增加，並可利用轉染及報告基因的方法證實 thrombin 及受體 N 端合成胜?SFLLRN-NH2(PAR1 作用劑)和 GYPGQV-NH2PAR4 作用劑)也可誘導 IL-8-luciferase 的活性，但 TFRGAP-NH2 PAR3 作用劑)卻無法增加 IL-8-luciferase 的活性。接著進一步證實，thrombin 誘導 IL-8 釋放及 IL-8-luciferase 的活性，可被轉染 dominant negative mutant of Rac (Rac N17)和 PI3K 抑制劑(LY 294002)及 dominant negative mutant of Akt (Akt DN)所抑制。Thrombin 具時間相性誘導 Rac 及 Akt 的活性，同時 thrombin SFLLRN-NH2 及 GYPGQV-NH2 也可誘導 Akt Ser473 的磷酸化。更進一步證實，thrombin 誘導 Akt Ser473 的磷酸化，可被 Rac N17 及 LY 294002 所抑制；同時 thrombin 所誘導 Akt 的激?活性，也可被 Rac N17、LY 294002 及 Akt DN 所抑制。此外，thrombin 所誘導的 IKKα/β的磷酸化可被 Rac N17、LY 294002 及 Akt 抑制劑所抑制；同時 Rac N17、LY 294002 及 Akt DN 可抑制 thrombin 誘導的 IKKα/β的激?活性。再者 RacN17、Akt DN 及 LY 294002 皆可以抑制 thrombin 誘導κB-luciferase 的活性。最後更進一步證實 thrombin 可以依時間相關性的誘導 Gβγ2 及 Rac 與 p85α的結合。綜合以上的實驗結果，可以推測出在 A549 肺部上皮細胞中，thrombin 可經由 Gβγ2/Rac/PI3K/Akt 的路徑活化 IKKα/β進一步再活化 NF-κB 來調控 IL-8 的表現及釋放。

英文摘要

Thrombin, a multifunctional serine protease generated at sites of vascular injury, and known for its pivotal role in the coagulation cascade, contributes to tissue repair, but also promotes a wide range of cellular responses including modulation of the inflammatory responses. A previous report showed that thrombin can induce IL-8 release in human epithelial cells, however, the signal transduction is still unclear. This investigated the signaling pathway involved in Gβγ2, Rac and PI3K/Akt in IL-8 expression caused by thrombin in A549 lung epithelial cell.Thrombin caused a concentration-dependent increase in IL-8 release, which was attenuated by cell transfection with dominant negative mutant of Rac (Rac N17), LY 294002 (a PI3K inhibitor), and dominant negative mutant of Akt (Akt DN). Thrombin, SFLLRN-NH2 (a PAR1 agonist peptide), and GYPGQV-NH2 (a PAR4 agonist peptide), all induced an increase in IL-8-luciferase activity, while TFRGAP-NH2 (a PAR3 agonist peptide) had no effect. Treatment of A549 cells with thrombin caused increase in Rac and Akt activities. Similarly, SFLLRN-NH2, and GYPGQV-NH2 but not TFRGAP-NH2 inducedphosphorylation of Akt at Ser473. Pretreatment of A549 cells with LY294002 or transient transfection with Rac N17 inhibitedthrombin-induced Akt activity. In addition, Rac N17 and Akt DN inhibited thrombin-induced IKKα/β kinase activity. Moreover, Rac N17,LY 294002, and Akt DN all inhibited thrombin-induced increase in κB-luciferase activity. Further studies reveals that thrombin induced therecruitment of p85α and Rac to Gβγ2 in time-dependent manner. These results indicate that thrombin activates the Gβγ2/Rac/PI3K/Akt signaling pathway to activate IKKα/β, which in turn initiates NF-κB activation,and ultimately induces IL-8 expression and release in A549 cells.