

c-Src Mediates Thrombin-Induced NF- κ B Activation and IL-8/CXCL8 Expression in Lung Epithelial Cells

林佳靜

Lin CH;Cheng HW;Hsu MJ;Chen MC;Lin CC;Chen BC

Abstract

In this study, we examined the regulation of NF- κ B activation and IL-8/CXCL8 expression by thrombin in human lung epithelial cells (EC). Thrombin caused a concentration-dependent increase in IL-8/CXCL8 release in a human lung EC line (A549) and primary normal human bronchial EC. In A549 cells, thrombin, SFLLRN-NH₂ (a protease-activated receptor 1 (PAR1) agonist peptide), and GYPGQV-NH₂ (a PAR4 agonist peptide), but not TFRGAP-NH₂ (a PAR3 agonist peptide), induced an increase in IL-8/CXCL8-luciferase (Luc) activity. The thrombin-induced IL-8/CXCL8 release was attenuated by D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (a thrombin inhibitor), U73122 (a phosphoinositide-phospholipase C inhibitor), Ro-32-0432 (a protein kinase C a (PKCa) inhibitor), an NF- κ B inhibitor peptide, and Bay 117082 (an I κ B phosphorylation inhibitor). Thrombin-induced increase in IL-8/CXCL8-Luc activity was inhibited by the dominant-negative mutant of c-Src and the cells transfected with the KB site mutation of the IL-8/CXCL8 construct. Thrombin caused time-dependent increases in phosphorylation of c-Src at tyrosine 416 and c-Src activity. Thrombin-elicited c-Src activity was inhibited by Ro-32-0432. Stimulation of cells with thrombin activated I κ B kinase $\alpha\beta$ (IKK $\alpha\beta$), I κ B α phosphorylation, I κ B α degradation, p50 and p65 translocation from the cytosol to the nucleus, NF- κ B-specific DNA-protein complex formation, and κ B-Luc activity. Pretreatment of A549 cells with Ro-32-0432 and the dominant-negative mutant of c-Src DN inhibited thrombin-induced IKK $\alpha\beta$ activity, κ B-Luc activity, and NF- κ B-specific DNA-protein complex formation. Further studies revealed that thrombin induced PKCa, c-Src, and IKK $\alpha\beta$ complex formation. These results show for the first time that thrombin, acting through PAR1 and PAR4, activates the phosphoinositide-phospholipase C/PKCa/c-Src/IKK $\alpha\beta$ signaling pathway to induce NF- κ B activation, which in turn induces IL-8/CXCL8 expression and release in human lung EC.