c-Src mediates thrombin-induced NF-kB activation and interleukin-8 expression in lung epithelial cells

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Abstract

In this study, we examined the regulation of NF-kappaB 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 kinsase C alpha (PKC alpha) inhibitor), an NF-kappaB inhibitor peptide, and Bay 117082 (an IkappaB 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 kappaB 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 IkappaB kinase alphabeta (IKK alphabeta), IkappaB alpha phosphorylation, IkappaB alpha degradation, p50 and p65 translocation from the cytosol to the nucleus, NF-kappaB-specific DNA-protein complex formation, and kappaB-Luc activity. Pretreatment of A549 cells with Ro-32-4032 and the dominant-negative mutant of c-Src DN inhibited thrombin-induced IKK alphabeta activity, kappaB-Luc activity, and NF-kappaB-specific DNA-protein complex formation. Further studies revealed that thrombin induced PKC alpha, c-Src, and IKK alphabeta complex formation. These results show for the first time that thrombin, acting through PAR1 and PAR4, activates the phosphoinositide-phospholipase C/PKC alpha/c-Src/IKK alphabeta signaling pathway to induce NF-kappaB activation, which in turn induces IL-8/CXCL8 expression and release in human lung EC.