SUMMARY OF Ph.D. DISSERTATION

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Title

Mechanistic studies and regulation by bioactive metabolites of NF-κB activation pathway

Abstract

TRAF6 transduces act downstream of the Toll/IL-1 receptors by interacting with IRAK-1 upon IL-1 stimulation. However, the mechanisms underlying regulation of IRAK-1/TRAF6 interaction are largely unknown. I have identified a novel TRAF-interacting protein, TIFA. In transient transfection assay, TIFA activates NF-κB and JNK. However, mutation that abolishes TRAF6 binding or mutations in the FHA domain failed to activate NF-κB and JNK. Overexpression of TIFA showed interaction between TRAF6 and IRAK-1. Analysis of endogenous proteins also indicated that TIFA associated with TRAF6 constitutively. It was also shown to be associated with IRAK-1 in an IL-1-stimulated cells. Thus, TIFA is likely to mediate IRAK-1/TRAF6 interaction upon IL-1 stimulation.

Bone destruction is often observed in advanced case of rheumatoid arthritis and neoplastic diseases including multiple myeloma. Effective and non-toxic chemotherapeutic agents are expected for suppression of these bone destructions. RANK induces activation of NF-κB and osteoclastogenesis in bone marrow-derived macrophages. (-)-DHMEQ, a novel NF-κB inhibitor, strongly inhibited RANKL-induced NF-κB activation in bone marrow-derived macrophages and inhibited RANKL-induced osteoclast differentiation. Interestingly, (-)-DHMEQ specifically inhibited the RANKL-induced NFATc1 expression, but not TRAF6 and c-Fos expressions. Inhibition of osteoclast formation by (-)-DHMEQ was rescued by overexpression of NFATc1, suggesting that the inhibition is not due to toxic effect. Moreover, pit formation assay showed that (-)-DHMEQ inhibited the bone-resorbing activity of osteoclasts. In addition, (-)-DHMEQ inhibited LPS-induced bone destruction model *in vivo*. Thus, NFATc1 expression was shown to be regulated by NF-κB, and (-)-DHMEQ is expected to become a new thrapeutic strategy against bone erosion. In conclusion, I found a new signal transducer TIFA for NF-κB activation, and using (-)-DHMEQ, I found new role of NF-κB in RANK signaling pathways.