【P-14】

Src Tyrosine Kinase Inhibitor PP2 Markedly Enhances Ras-Independent Activation of Raf-1 Protein Kinase by PMA and Hydrogen Peroxide

Young Mi Lee, Ji-Young Kim, Mi-Young Hong and Michael Lee
Laboratory of Genetic Toxicology, Korea Institute of Toxicology, KRICT, Daejeon, Korea

Recently we reported that simultaneous treatment of NIH 3T3 cells with the combination of phorbol myristate acetate (PMA) and hydrogen peroxide resulted in synergistic activation of Raf-1 kinase. In the present study, we have demonstrated that PP2 (4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine), a potent and selective inhibitor of the Src-family tyrosine kinase, greatly potentiated the ability of PMA and/or hydrogen peroxide to activate Raf-1 kinase while it blocked the tyrosine phosphorylation of Raf-1. Unlike PMA/hydrogen peroxide treatment, which showed transient activation, PP2-mediated Raf-1 activation was sustained and continued to increase through 4 h of treatment. Transient transfection studies with a dominant-negative mutant of Ras indicated that this PP2-induced activation of Raf-1 was Ras-independent. Moreover, PP2 showed no effect on platelet-derived growth factor (PDGF)-induced Raf-1 activation. Interestingly, mutation of the reported Raf-1 Src family tyrosine kinase phosphorylation site by conversion of tyrosines 340 and 341 to phenylalanine (YY340/341FF Raf) had limited effect on the ability of PP2 to induce significant stimulation of Raf-1 kinase activity. Taken together, our results suggest that a tyrosine phosphorylation event is involved in the negative feedback regulation of Raf-1. Inhibition of a Src family tyrosine kinase by PP2 appears to alleviate this tyrosine kinase-mediated inhibition of Raf-1 and allow activating modification(s) of Raf-1 to proceed. This PP2 effect resulted in significant and sustained Ras-independent activation of Raf-1 by PMA and hydrogen peroxide.

Keyword: Raf-1, Src, PP2, hydrogen peroxide, PMA