Involvement of Ras/Raf-1/Erk actions in the magnolol-induced upregulation of p21 and cell cycle arrest in colon cancer cells.

李文森

Hsu YF;Lee TS;Lin SY;Hsu SP;Juan SH;Hsu YH;Zhong WB;Lee WS

Abstract

Previously, we showed that magnolol induces cell-cycle arrest in cultured colon and liver cancer cells through an upregulation of the p21 protein [1]. The aim of this study was to delineate the molecular mechanism underlying this magnolol-induced increase of p21 protein. Thus our RT-PCR analysis demonstrated that the mRNA levels of p21 were increased at 1 h after magnolol treatment and sustained for at least 24 h. The p21 promoter activity was also increased by magnolol treatment. Western blot analysis demonstrated that treatment of COLO-205 cells with magnolol increased the levels of phosphorylation of extracellular signal-regulated kinase (ERK). Pretreatment of the cells with PD98059 abolished the magnolol-induced upregulation of p21 protein, suggesting the involvement of an ERK pathway in the magnolol-induced upregulation of p21 in COLO-205 cells. Ras inhibitor peptide abolished the magnolol-induced increase of phosphorylated ERK protein levels, increase of p21 protein, and decrease of thymidine incorporation. Moreover, treatment of COLO-205 with magnolol increased the phosphorylated Raf-1 protein (the Ras target molecule). Pretreatment of the cells with Raf-1 inhibitor reversed the magnolol-induced decrease in thymidine incorporation. Treatment of the cells with CaM kinase inhibitor, but not protein kinase A (PKA) inhibitor or phosphatidylinosital 3-kinase (PI3K) inhibitor, abolished the magnolol-induced activation of ERK and decrease of thymidine incorporation. Taken together, our results suggest that magnolol activates ERK phosphorylation through a Ras/Raf-1-mediated pathway. Subsequently, p21 expression is increased, and finally thymidine incorporation is decreased.