RhoA modification and its effect on prostate cancer cell death

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**Background** Rho A is a small GTP-binding protein which controls cell adhesion and motility through organization of the actin cytoskeleton and regulation of actomyosin contractility. To determine whether blocking RhoA geranylgeranylation accounts for drug-induced apoptosis, we used geranylgeranyl pyrophosphate to compensate for the inhibition of geranyl group synthesis pathway, and herein demonstrate a correlation between inhibition of protein geranylgeranylation and tumor cell apoptosis. Lovastatin is a fungal product and act as a competitive inhibitor of HMG-CoA reductase which results in deprivation of isoprenyl groups from a variety of post-translationaly modified protein products, such as RhoA, in the cell.

**Methods** Human prostate cancer cell line PC-3 was used. FACS analysis was performed to see the cell cycle distribution. Western blot analysis for RhoA, Immunohistochemical analysis for intracellular distribution of RhoA. Cell death was studied using DNA ladder formation and caspase activity analysis. Cell growth was monitored by MTT assay.

**Results** We show that the growth of PC-3 was differentially regulated by RhoA signal transduction system. Transfection of constitutive active RhoA demonstrated more rapid growth comparing to cells transfected with wild-type RhoA or dominant negative RhoA. Membrane translocation of RhoA is blocked by lovastatin, suggesting that inhibition of geranylgeranylation may be important in RhoA-mediated cell proliferation. We found that blocking protein geranylgeranylation with lovastatin was followed by an apoptosis in a time- and dose-dependent manner. Cell death induced by lovastatin required 36 to 96 hr depending on the cell line tested. In lovastatin-treated cells, PARP was degraded, confirming the occurrence of apoptosis. To better understand the cell death mechanism caused by lovastatin, DNA ladder formation and caspase activity was investigated as well.

**Conclusion** These studies suggest that inhibition of post-translational modifications such as geranylgeranylation of the RhoA may influence prostate cancer cell proliferation. Further investigations are in progress to elucidate the mechanism of action and the possible therapeutic value of this treatment.