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Role of the calcium-calmodulin dependent kinase II in oncogenic Ras-induced proliferation

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Activation of the Ras/ERK pathway and stimulation of proliferation by integrins in thyroid cells requires CaMKII activation. To date, whether this role of CaMKII is a general mechanism or is restricted to integrin signalling is unknown. As oncogenic activation of the Ras/ERK pathway by mutated Ras, RET/PTC and Trk is frequent in papillary thyroid carcinoma (PTC), we investigated the expression and activation level of CaMKII in PTC primary culture and in stable cell lines.

The level of activation of CaMKII and ERK was determined by Western blot with anti-phosphorylated antibodies and by *in vitro* kinase activity assay. In 5 primary cultures and in cell lines starved from serum and left in suspension for 30 min, CaMKII and ERK remained activated while were inhibited by calmodulin inhibitors. CaMKII inhibitors blocked ERK phosphorylation. These results indicate that constitutive up-stream signals activate CaMKII and that this kinase is necessary to ERK activation in PTC cells.

To determine whether oncogenic Ras induced CaMKII activation, NIH-3T3 were transfected with plasmids encoding recombinant mutated H- and K-Ras^{V12} for transient expression. In NIH-3T3 cells starved from serum, CaMKII was not active. Oncogenic Ras induced CaMKII activation inhibited by calmodulin inhibitors. Stimulation of ERK activation and [^{3}H]thymidine incorporation by oncogenic Ras were completely suppressed by CaMKII inhibitors. These results indicate that oncogenic Ras induces CaMKII activation through modulation of intracellular calcium concentration and that this kinase is necessary to stimulate cell proliferation. As the activation of the Ras/ERK pathway is a major mechanism of sustained proliferation in many tumors, CaMKII might represent a novel pharmacological site of intervention in the treatment of cancer.

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