

Differential effects of Ha-RasG12V, Ki-RasG12V and Ki-RasG13D on cell proliferation

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Although differing only for the last 24 aminoacids, the three major isoforms of p21 Ras (Ha-, Ki- and N-Ras) can trigger alternative pathways of signal transduction, at least in part as a consequence of different post-translational modifications and subcellular localization. Ras mutations are a common event in tumorigenesis. In colorectal carcinomas (CRCs) the mutations affect almost exclusively Ki-Ras, while Ha-Ras mutations are mostly found in bladder carcinomas and N-Ras mutations in leukemia cells. In almost all cases, the genetic alteration is a point mutation in codons 12 or 13, and less frequently in codon 61. By affecting the GTPase activity of the protein, they always lead to a constitutively active protein. However, data obtained in different experimental systems or by analysis of primary and metastatic tumors show that mutations in different codons, different mutations in the same codon, and mutations of different isoforms of Ras may have diverse biological consequences. To shed more light on the molecular mechanisms responsible for the different effects of Ras mutations, we have obtained stable clones of HT-29 (a human colorectal adenocarcinoma cell line in which the endogenous Ras genes are wild type) transfected with cDNAs codifying Ha-RasG12V, Ki-RasG12V and Ki-RasG13D, under the control of an hormone-inducible promoter. The expression of each of these mutated Ras isoforms induces specific, different effects on cell morphology and growth rate. FACS analysis shows also a differential effect on the cell cycle. H-RasG12V expression, in addition, seems to determine apoptosis. Despite these differences, all three mutated isoforms of Ras increase the expression of the CDK inhibitor p21. Preliminary data reveal epigenetic changes in the p21 promoter region upon induction of H-RasG12V expression.