Analysis of the mechanisms through which K-RASG12V and K-RASG13D regulate the proliferation and cell death in cells HT-29.

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The three major isoforms of RAS (H-, K- and N-RAS) differ only in the last 25 amino acids which are the site of different post-translational modifications that lead to localization in diverse plasma membrane microdomains and activation of alternative pathways of signal transduction. This might explain, at least in part, the different biological effects of the RAS isoforms in the cells. RAS mutations are a common event in several tumours and in colorectal carcinomas the genetic alteration is a point mutation in codons 12 or 13 of K-RAS. These mutations lead to a constitutively active protein by inactivating its GTPase activity. To shed more light on the molecular mechanisms responsible for the different effects of RAS mutations, we have used stable clones of HT-29 (a human colorectal adenocarcinoma cell line in which the endogenous RAS genes are wild type) transfected with cDNAs codifying: K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of a Mifepristone-inducible promoter [GeneSwitchTM System (Invitrogen)]. We found that the two mutated isoforms of K-RAS induce different effects on the growth rate and on the cell cycle and a similar increase in the $p21^{CIP1/WAF1}$ expression. The increase of $p21^{CIP1/WAF1}$ protein expression and the effects on the cell cycle are not reduced by treatment with MEK or/and PI3K inhibitors. K-RASG12V and K-RASG13D also induce differential effects on the pro-apoptotic (MST2-RASSF1A-LATS1) and the anti-apoptotic (MST2-RAF-1) pathways. Finally, the data show an increase in total cell death in both induced clones, but K-RASG12V induce a significant increase in apoptosis while K-RASG13D in necrosis; the cytotoxic effect observed in induced K13 cells decreases in starved conditions.