Distinct biological effects are observed in HT-29 colorectal carcinoma cells induced to express K-RASG12V or K-RASG13D

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RAS are small membrane-bound GTPase proteins involved in signalling pathways that regulate proliferation, differentiation and apoptosis in all cell types, and whose activating mutations have oncogenic effects. The three major isoforms of p21 RAS (H-, K- and N-RAS) have a high degree of homology, especially in the regions involved in interactions with GDP/GTP and with regulatory proteins and effectors, but differ significantly in the C-terminal

25 amino acids region. This hypervariable domain is the site of post-translational modifications specific for each isoform, which result in distinct intracellular trafficking routes and final subcellular localizations, where the type and concentration of regulators and effectors may differ.[1] This may explain the observed non-overlapping functions of these proteins, the different biological effects of their physiological activation, and their differential involvment, when mutated, in specific tumor types. In almost all cases, the genetic alterations detected in tumoral cells are missense point mutations in codons 12 or 13, more rarely in codon 61, and they always result in a constitutively active protein by inactivating its GTPase activity. Mutations in the K- RAS isoform are a frequent, early event in colorectal tumorigenesis and their occurrence is considered to be a resistance factor to therapies based on anti-EGFR monoclonal antibodies.[2]

However, molecular epidemiological studies in different primary and metastatic tumors suggest that mutations in different codons or different mutations in the same codon of Ras may have diverse biological consequences[3] and may lead to a different response to drug treatments. In particular, one of the new drugs developed for the treatment of colorectal carcinoma is Cetuximab, a monoclonal chimeric human/mouse antibody IgG1, which acts against the extracellular domain of EGFR. The binding of this antibody to the receptor causes a direct inhibition of its tyrosine kinase activity resulting in the inhibition of several pathways of signal transduction mediated by RAS, such as those of PI3K/AKT, and RAF/MAPKs. This stimulates pro-apoptotic mechanisms and the inhibition of cell proliferation. Several clinical trials conducted in recent years have shown that patients with CRC who have mutations in K-ras codon 12 or 13 respond heterogeneously to Cetuximab treatment and for this reason are currently excluded from treatment with this drug. However, it has recently been reported that tumors bearing the K-RASG13D mutation may show some response to the therapy.[4] It is also currently unclear whether mutations in BRAF (an effector of RAS) affect the response to Cetuximab.[2]

To shed more light on the molecular mechanisms responsible for the different effects of Ras mutations, we established an experimental system by isolating stable clones of HT-29 cells (a human colorectal adenocarcinoma cell line characterized by mutations in the BRAF and PIK3CA genes and 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. Cell proliferation assays and cytofluorimetric analysis reveal that activation of the expression of K-RASG12V and of K-RASG13D have distinct biological effects on the cells. We have also analysed the response of the induced and not induced cells to treatment with inhibitors of the two main RAS effectors (MEK and PI3K) and with the anti-EGFR monoclonal antibody Cetuximab.

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