Analysis of the effects of K-RasG12V and K-RasG13D on the cell cycle

M.R. Saladino, I.Albanese
Diparimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO)-Università degli Studi di Palermo

p21 Ras is small protein with GTPase activity that regulates proliferation, differentiation and apoptosis in all cell types. The three major isoforms of Ras (H-, K- and N-Ras) differing only for the last 24 aminoacids have different post-translational modifications that lead to localization in diverse plasma membrane microdomains and downstream 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 almost all cases they are point mutations in codons 12 or 13, and rarely in codon 61. These mutations lead to a constitutively active protein by inactivating the GTPase activity. However, data show in different primary and metastatic tumors that not only mutations of different isoforms of Ras, but also mutations in different codons or different mutations in the same codon of the same isoform of Ras have diverse biological consequences. In particular, in colorectal carcinomas (CRCs) Ras mutations are quite frequent and affect mainly K-Ras, usuallly already at a early stage of tumor development. 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. We found that the expression of K-Ras mutated in two different codons (codon 12 or codon 13) induces specific and different effects on the growth rate. Cytofluorimetric analysis shows also a differential effect on the cell cycle. Finally, Western analysis shows a significant increase in the expression of the cell cycle inhibitor protein p21 in response to induction of K-RasG12V or K-RasG13D expression. Whether the regulation of the CDK inhibitor p21 expression occurs through MAPK or PI3K signalling pathways is presently under investigation.