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1 O

EXPERIMENTAL MALIGNANT CONVERSION OF EARLY STAGE HUMAN MELANOMA CELLS: EVIDENCE FOR A DOMINANT PHENOTYPE

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By virtue of insertional mutagenesis with a replication defective retrovirus, we have recently succeeded in isolating tumorigenic variants from biologically benign non-tumorigenic human cuaneous melanoma cells. Unlike the parental cells, from which they have been derived, the aggressive variants expressed a cluster of biological characteristics described to be associated with clinically malignant lesions. In addition to tumor forming ability in nude mice, their in vitro survival and growth were anchorage and growth factor independent, they over-expressed the MUC18 adhesion molecule and they lost responsiveness to the growth inhibitory effect of cytokines. The inheritable presence of provirus stably integrated in distinct and well defined loci of the genome co-segregated dominantly with the above phenotypes. The possibility exists that specific genes targeted by the inserted provirus may play a causative role in the progression of the described experimental model as well as natural progression of the disease. The identification of the affected genes is currently under investigation.

3 O

REENTRY INTO THE CELL CYCLE OF UVc-IRRADIATED QUIESCENT NORMAL HUMAN DIPLOID FIBROBLASTS: ITS RELATIONSHIPS WITH P53 INDUCTION OR INHIBITION.

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In order to assess the possible role of p53 in response to DNA damage occuring in normal quiescent cells, human diploid fibroblasts in confluence for more than two weeks were exposed to UVc irradiation (20 J/m^2), with and without p53 antisens oligonucleotides, and time-course cell cycle analysis was performed by flow cytometry for the next 72 hrs.
The accumulation of p53 in these irradiated cells at 24th and the inhibition of this induction by p53 antisens oligonucleotides were verified by ELISA.
After reseeding of control unirradiated fibroblasts, reentry into the cell cycle occured at the 48th hr. In UVc-irradiated cells, absolutly no reentry into the cell cycle was observed.
In quiescent irradiated fibroblasts in which p53 accumulation was inhibited by p53 antisens, a reentry into the cell cycle appeared as soon as the 24th, i.e even before unirradiated control cells.
These results show that in confluent normal human diploid fibroblasts, the induction of p53 by a DNA damaging agent creates a complete block and inhibits reentry into the cell cycle. The inhibition of p53 induction not only overcomes this block, regardless of DNA damages, but also permits an earlier reentry into the cell cycle.
In conclusion, we demonstrate that p53 protein inhibits the reentry into the cell cycle of quiescent normal human fibroblasts exposed to DNA damaging agents. This suggests that p53 could induce a G0/G1block, in addition of a G1/S block which is moreover controversed. To clearly evaluate if this block is a G0/G1 or a G1/S block some more experiments are going on, including p27^{kip1}, p21$^{waf1/cip1}$ and cyclin D expression studies.

2 P

RELATIONSHIP BETWEEN rTNFα-, APOPTOSIS- AND MULTIDRUG-RESISTANCE IN VITRO.

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Expression of both P-glycoprotein (P-gp) and multidrug-resistance-related protein (MRP) confers resistance to antineoplastic drugs (multidrug-resistance; MDR). Analogous to the MDR-phenomenon, tumor cells can also develop resistance to toxic impacts of TNFα (cytokine resistance). We investigated the relationship between these resistance types using either P-gp or MRP overexpressing cell lines (CCRF-CEM, HL-60, K562, KB, HeLa, LoVo, CHO, GLC4), selected by various drugs. Our data (MTT-assay) show that the drug-selection process can have an impact on rTNFα-responsiveness leading to both resistance and sensitivity even within the same cell model. A change in TNFα -responsiveness was 1) not dependent on P-gp or MRP-expression, 2) not defined by the cell type but 3) more likely a consequence of the selection process. By means of the CCRF-CEM leukaemia model, including both rTNFα-resistant and -sensitive MDR-sublines, we studied whether the modulation of parameters causing rTNFα-resistance (p55, endogenous TNFα, MnSOD, HSP-70, HSP-27) or apoptosis-resistance (bcl-2, bcl-xl, c-myc) parallels rTNFα-responsiveness (RT-PCR, Western blot). Additionally, sensitivity to other apoptosis-inducers (H_2O_2, UV-light) was investigated. Results give evidence for a role of endogenous TNFα and MnSOD in rTNFα-responsiveness. rTNFα-resistance in CCRF-CEM cells implies no general „apoptosis resistance phenotype". We conclude that MDR phenotype and rTNFα-responsiveness are not directly linked. The rTNFα-hypersensitivity of some MDR cell lines implicate new therapeutic strategies against drug-resistant tumors.

4 P

PROGNOSTIC VALUE OF MOLECULAR ALTERATIONS OF P53 AND K-RAS GENES IN NON-SMALL CELL LUNG CANCER.

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The potential value of K-ras and p53 mutations, as well as p53 immunohistochemistry, as prognostic indicators in NSCLC was studied. DNA was isolated from paraffin embedded tumors samples from a surgical series of 71 postoperative chemotherapy naive patients. K-ras genotypes were analyzed by PCR / allele-specific oligodeoxynucleotide (ASO) hybridization, and p53 disfunction was assayed by immunohistochemistry and by PCR / single-strand conformation polymorphism (SSCP) (exons 5-8) followed by DNA cycle sequencing. The presence of K-ras mutations (22%) and particularly those corresponding to aspartic acid and serine at codon 12, seemed to confer a worse prognosis. However, the presence of p53 mutations (34%) correlated with better survival (stage I; $p = 0.03$), while the accumulation of p53 protein did not affect the clinical outcome. The correlation between the presence of p53 sequence alterations and protein detection by immunohistochemistry was 58%. Only one tumor contained mutations in K-ras and p53 (exon 5) genes, indicating that those genes are involved in different NSCLC tumorigenesis pathways.