Molecular Basis of Drug Resistance

5.001

HUMAN O^6ALKYLGUANINE-DNA-ALKYLTRANSFERASE GENE EXPRESSION IN SOMATIC CELL HYBRIDS.

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Alkylating agents are widely used as anticancer drugs. A major DNA lesion given by alkylating agents is O^6alkGua. This lesion is repaired by a specialized enzyme, O^6alkylguanine-DNA-alkyltransferase (AT). This enzyme makes tumoral cells resistant to alkylating agents. The activity level differs widely among normal as well as tumoral cells. Using six human/hamster cell hybrids showing different AT activity we have investigated the expression of the human gene , to find out at which level the regulation is exerted. We observed that the human gene was expressed in all hybrids. The transcriptional level correlated with enzyme activity. One possible exception was represented by an hybrid with high messanger production and medium-low enzyme activity. Our data indicate that the control of the AT activity is at the transcriptional level.

5.002

ASSESSMENT OF ADRIAMYCIN (ADR) INCORPORATION IN HUMAN OSTEOSARCOMA (OSA) CELLS

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A new method has been recently suggested for the analysis of intracellular ADR incorporation as an index of chemosensitivity (ORS Trans, 14,1989). We have evaluated the reliability of this method on 6 primary cultures, 2 cell lines (U2-OS and Saos-2) and 2 ADR-resistant variants (U20S/DX and Saos-2/DX) of human OSA. The percentage of growth inhibition after in vitro ADR treatment, the nuclear ADR incorporation, and the expression of GP-170 were considered. All the primary cultures,as well as the U2OS and Saos-2 parental cell lines showed sensitive to ADR with a high inhibition of cell growth (median IG = 82%), high levels of nuclear ADR incorporation (median of sensitive cells = 92%) and costantly negative GP-170 expression. On the contrary, U2OS/DX and Saos-2/DX showed a <5% median IG, a < 5% median of sensitive cells and a high expression of GP-170 (median of positive cells = 84%). Our data confirm this assay as a promising tool for the clinical prediction of chemosensitivity.

5.003

IN VITRO INTRACELLULAR ACCUMULATION OF FCE 23762, A NEW ANTHRACYCLINE ACTIVE ON DX-RESISTANT TUMOR CELLS

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FCE 23762 (3'desamino-3'[2(S)methoxy-4-morpholinyl] doxorubicin) is a new derivative of Doxorubicin (DX) active in vitro and in vivo on DX-resistant cells.
FCE 23762 is highly lipophilic and is more potent than DX both in vitro and in vivo. We have evaluated in vitro the intracellular accumulation, and the kinetic of uptake and efflux of FCE 23762 and DX on a panel of DX-sensitive and DX-resistant cells, both of murine and human origin.
The results indicate that FCE 23762 is able to accumulate into tumor cells with a faster kinetic and to reach significantly higher levels than DX.
Of note, this compound reaches elevated intracellular contents also in all DX-resistant tumor cell lines tested.

5.004

Characterization and reversal of cisplatin resistance in a human larynx carcinoma cell line

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We have established four cisplatin (CDDP)-resistant sublines (DDP1 to DDP4) of the recloned laryngeal squamous carcinoma cell line HLac79-ML. Three sublines (DDP2,3,4) contained significantly elevated glutathione (GSH) levels, two sublines (DDP2,4) showed increased GSH-S-transferase activity, and DDP4 cells showed increased γ-glutamyltranspeptidase activity. CDDP uptake was reduced in DDP3 and 4 cells. GSH depletion with buthionine suloximine (BSO) led to a significant increase of CDDP sensitivity in all HLac79 subpopulations, whereas verapamil, cyclosporin A and aphidicolin were not effective. Combined BSO (30 mM suppl. in drinking water) and CDDP (3x3 mg/kg bw) treatment in HLac79 tumor bearing nude mice produced significant retardation of tumor growth as compared to chemotherapy alone.

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5.005

MULTIDRUG SENSITIVITY PHENOTYPE IN UNSELECTED HUMAN LUNG CANCER CELL LINES ASSOCIATED WITH TOPOISOMERASE II EXPRESSION. Capranico G., G. Giaccone*, M. Binaschi, Supino R., AF. Gazdar*, and F. Zunino. Istituto Nazionale Tumori, 20133 Milan, Italy, and *NCI-Navy Medical Oncology Branch, NIH, Bethesda, MD 20892, USA.

Drug sensitivities of human lung cancer cell lines unselected in vitro for resistance were studied in relation to topoisomerase (topo) II expression. These cell lines showed a multidrug sensitivity phenotype (MSP), as sensitivities to a number of cytotoxic drugs with different cellular targets were significantly correlated with each other. Marked differences were observed in topo II gene expression. The level of topo II expression correlated with the MSP, and nuclear topo II activity paralleled topo II expression. Thus, low levels of topo II expression predicted a reduced sensitivity of unselected human lung cancer cell lines to several drugs, including agents with different targets. It is proposed that regulations of a common pathway of cell death might influence cell sensitivity to different drugs, and that topo II could play an important role in the cell response to DNA damage produced by drug actions. Moreover, a 3'-end topo II gene rearrangement, leading to an abnormal mRNA and an enzyme with a lower DNA affinity was found in a cell line, which was poorly sensitive to drugs. The presence of a topo II with reduced DNA binding affinity might have consequences on the organization of the nuclear chromatin, and might contribute to the drug resistance of this cell line

5.006

Cell Cycle Kinetics and the Induction of Programmed Cell Death by Cytotoxic Agents in Tumour Cells. *Thomas G. Cotter, Jacqueline M. Glynn and Douglas R. Green, *Dept. of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland and Division of Cellular Immunology, La Jolla Institute for Allergy and Immunology, 11149 N. Torrey Pines Road, La Jolla, CA 92037.

This study demonstrates that three cytotoxic agents with distinct mechanisms of action all kill murine tumour cells by induction of programmed cell death (apoptosis). We proposed to test whether the phase of the cell cycle in which a cell is in has a bearing on how readily it undergoes programmed cell death (apoptosis). Elutriated or chemically synchronised murine T cell hybridoma cells (A1.1) were induced to undergo apoptosis by treatment with the cytotoxic agents, actinomycin D 1ug/ml, aphidocolin 1ug/ml or camptothecin 200 ng/ml. Cell death was quantified by both ^{3}H-thymidine release and MTT assays. Agarose gel electrophoresis was used to demonstrate endonuclease mediated internucleosomal DNA and confirm that cell death was via apoptosis. All three cytotoxic drugs induced extensive apoptosis in G1, S and G2/M phases of the cell cycle. Cells blocked and held in mitosis by treatment with the microtubule disrupting agent nocodazole also appeared to die via apoptosis following exposure to the cytotoxic agents.This indicates that cells in this phase of the cell cycle which have no nuclear membrane and highly condensed chromatin are capable of undergoing apoptosis. Cell death by these agents did not require RNA or protein synthesis. These data indicate that activation of a specific endonuclease is a critical event in cell death induced by camptothecin, actinomycin D and aphidocolin and this can occur in each phase of the cell cycle. Furthermore, the above results have a direct bearing on our understanding on how cytotoxic agents exert their effects.