Symposium no. 5: Molecular Basis of Drug Resistance

5.013

INTRACELLULAR DRUG ACCUMULATION OF A PROGESTIN AND AN ANTIESTROGEN IN MULTIDRUG RESISTANT HUMAN BREAST CANCER CELLS. F.Leonessa,J.Lippman, and R.Clarke. V.T.Lombardi Cancer Research Center, Georgetown University Medical Center, Washington,D.C. 20007, U.S.A.

The majority of agents which reverse multidrug resistance conferred by the MDR1 glycoprotein compete with cytotoxic drug for efflux out of the cell by the glycoprotein itself. We wished to determine the ability of MDR1 to alter the intracellular transport kinetics of hormones which can reverse resistance, i.e. an antiestrogen and a progestin. Consequently, we have measured the cellular uptake of tamoxifen and the synthetic progestin ORG2058 in the parental and multidrug resistant variants of MCF7 human breast cancer cells. Our data clearly indicate that overexpression of the P170 glycoprotein did not alter tamoxifen transport, but significantly reduced transport of the progestin (by up to 70 %). These results suggest that the progestins may reverse drug resistance by directly competing with cytotoxic drugs for transport by the the P170 glycoprotein. The effects of tamoxifen is may be related to a noncompetitive interaction.

5.014

Increased damage recognition proteins in cis-platin resistant cells. **Karen McLaughlin & Robert Brown. CRC Dept Medical Oncology, Beatson Laboratories, Glasgow G61 1BD**

We have detected proteins of approximate relative molecular mass 25, 50 and 100kd in human cell extracts that can bind to DNA which has been treated in vitro with the chemotherapeutic drug cis-diammine-dichloroplatinum (II) (cis-DDP). The 50kD and 100kD cis-DDP damage recognition proteins (DDRPs) are increased in a cis-DDP resistant ovarian human tumour cell line compared to the parental sensitive line. Binding of the proteins to the cis-DDP treated DNA can be competed with DNA containing cis-DDP adducts, but not by unplatinated DNA. Single-stranded DNA also binds to proteins of approximate molecular mass 50 and 100kd, which are increased in the cis-DDP resistant cell line, suggesting these are identical to the DDRPs. The 25kd DDRP shows no detectable binding to single-stranded DNA. Binding of these proteins to cis-DDP damaged DNA and the increased binding activity detected in cis-DDP resistant cells suggests that alteration in these proteins may play a role in cis-DDP resistance. At present we are examining these DDRPs in a variety of cell lines of known sensitivity to cis-DDP and in human tumour biopsy samples.

5.015

PHENOTHIAZINES REVERSE DOXORUBICIN RESISTANCE.
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Phenothiazines have cell surface actions and calmodulin-altering activity. We therefore investigated whether they had any effect on cell growth or resistance to growth inhibition by doxorubicin (DOX) using the HTC rat hepatoma cell line. HTC cells grew normally in DOX 0.2μM but had 50% growth inhibition at 0.4μM. By contrast, prochlorperazine (Compazine) 3μg/ml had a minimally inhibitory growth effect alone, but caused a 70% inhibition of growth in the presence of DOX 0.2μM. Similar antagonism of resistance to DOX was found for trifluroperazine (Stelazine) and thioridazine (Melleril) but not promethazine (Phenergan). No effects on drug resistance were found for the related Butyrophenones or Antihistamines. Prochlorperazine was also found to inhibit the expression of Mdr-1 mRNA in these cells. These data show effects on reversal of drug resistance by a whole class of clinically available drugs.

5.016

Determination of low-level P-glycoprotein expression by flow-cytometry

Müller, M.R., Boogen,C., Lennartz, K., Nowrousian, M.F., Rajewski, M.F., Seeber, S.

We have compared flow-cytometry and immunohistochemistry for the determination of P-glycoprotein (PGP) expression in cell lines and leukaemic blasts from patients with acute myeloid leukaemia (AML) using the monoclonal antibody MRK 16. A clear distinction in staining was observed between cells of the drug sensitive parental human lung cancer cell line H69/P and its multidrug resistant counterpart LX4. Low level antigen expression was detected in blasts from a number of AML. In order to improve the signal to background ratio, a histogram subtraction analysis was applied which is based on a statistical test for the calculation of the fraction of immunostained cells. Using this method, subpopulations of leukaemic cells with low expression of PGP could be detected in 10 out of 26 cases. We conclude that the flow-cytometric method is suitable for the detection of low-level PGP expression.

5.017

MOLECULAR AND CELLULAR BASIS OF DRUG RESISTANCE TO TIAZOFURIN (TR), AN INDUCER OF CANCER CELL DIFFERENTIATION
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IMP dehydrogenase, the rate-limiting enzyme of de novo guanylate biosynthesis provides sensitive target in cancer chemotherapy (Weber,Ca.Res.43:3466,1983).In K562 human leukemia cells the activity of IMP dehydrogenase (IMP DH) markedly increased.Tiazofurin (TR) through its active metabolite, TAD, inhibited IMP DH. In K562 human leukemia cells TR induced erythroid differentiation. Concurrently, a correlation was demonstrated between intracellular GTP concentration and expression of c-myc and H-ras genes in K562 cells induced to differentiate (Oláh,et al.:PNAS,85:6533,1988). A 1500-fold resistance to TR was induced in K562 cells. In resistant cells an increased IMP DH activity, and elevated GTP concentration were seen, while the inhibitory effect of TAD was decreased. An atypical multidrug resistance (MDR) was induced by TR. MDR phenotype was associated with elevated expression of mdr-1 gene (Northern-blot, PCR) however, gene amplification was not seen. The cellular and molecular mechanisms to overcome TR induced resistance is a key area of our present studies. (Supported by OTKA)

5.018

CHARACTERIZATION OF THE CHINESE HAMSTER V79 CELLS IRRADIATED WITH MULTIPLE GAMMA RAYS FRACTIONS
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We had found previously that Chinese hamster V79 cells exposed to multiple fractions of gamma rays become resistant to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and vincristine sulfate without changing the sensitivity to acute doses of gamma rays or UV light, as determined through survival assay and mutation induction. Here we report that preirradiated cells have increased levels of glutathione and metallothioneins, and, very likely, increased activity of plasma membrane P-glycoprotein. The expression of c-myc oncogene was found to increase linearly with the total accumulated dose of gamma rays. Increased resistance to MNNG as well as increased expression of c-myc oncogene were observed also 20 and 30 days after the last daily dose of gamma rays. Our results indicate that multiple irradiations with gamma rays induce numerous and long-lasting changes in preirradiated Chinese hamster V79 cells.