Proffered papers

Drug resistance and pre-clinical drug development—new targets

105 ORAL

CORRELATION BETWEEN EXPRESSION OF MDR1, GST-π, K-RAS, MDR3, CEA IN HUMAN TUMOR XENOGRAFTS

C. Weissenberger, S. Heck, D.P. Berger, L. Herbstritt, S. Schulzke, W.A. Dengler, R. Mertelsmann, H.H. Fiebig
University of Freiburg, Department of Internal Medicine I, D-79106 Freiburg, Germany

The drug resistance due to the overexpression of MDR1 is a major obstacle to a successful chemotherapy of human malignancies. In order to survey the mechanisms of regulation of MDR1 the expression of various tumor characteristics were studied in 62 different solid human tumor types growing subcutaneously in nude mice (xenografts). Northern and slot blot analysis revealed a statistical significant correlation ($P < 0.05$) between MDR1 expression and expression of glutathione-S-transferase (GST-π), k-ras, MDR3, but not to the expression of p53. CEA-positive tumors showed a significant higher expression of MDR1 ($P < 0.05$) and GST-π ($P < 0.01$) than CEA-negative tumors. GST-π showed a highly significant correlation ($P < 0.001$) with c-myc, k-ras and a significant correlation with p53 ($P < 0.05$). Additionally, a relationship between mdr3 expression, the expression of the EGF receptor and k-ras was observed ($P < 0.05$) as well as a weak association with the chemosensitivity to doxorubicine ($P < 0.10$). No other significant correlation between MDR1, MDR3 or GST-π expression and the chemosensitivity to 14 tested anticancer drugs could be observed, indicating that drug resistance cannot be explained solely on the basis of the expression of these resistance factors.

In conclusion, the association of MDR1, MDR3 and GST-π with oncogenes (k-ras) as well as with other tumor characteristics (CEA, p53) implies the involvement of the regulation of these resistance factors in complex molecular events during the progression of human cancers.

106 ORAL

CHEMOSENSITISATION OF HUMAN ADENOCARCINOMA CELLS BY ANTISENSE AGAINST THE MULTIDRUG RESISTANCE (MDR1) GENE

P.J. Roberts, J.I. Penny[1], F.C. Campbell[1]
Departments of Surgery, Helsinki University Central Hospital, Finland
[1]Ninewells Hospital, Dundee, U.K.

The MDR1 gene encodes P-glycoprotein (Pgp) which pumps cytotoxic agents out of cells and thus induces chemoresistance. Inhibition of this gene could impair Pgp expression and sensitise the cells to cytotoxic drugs. Pgp expression (by Western blot), sensitivity to doxorubicin (by MTT assay) and efflux of the Pgp mediated substrata rhodamine were investigated in human (Hap 2A) adenocarcinoma cells before and after treatment by antisense (AS) and sense (S) oligonucleotides against MDR1.

Both AS1 (against initiation region) and AS2 (against loop forming site) significantly (approx. 50%) inhibited Pgp expression and gave a 10-fold increase in chemosensitivity to doxorubicin. Both AS1 and AS2 inhibited rhodamine efflux. AS2 appeared more effective in all the tests. PgP expression returned to normal within 5 days of withdrawal of AS. Sense oligonucleotides S1 and S2 were ineffective.

Conclusions: MDR1 antisense inhibits Pgp expression and sensitises human adenocarcinoma cells (Hep 2A) to doxorubicin chemotherapy.

107 ORAL

HETEROGENEITY IN DNA-DAMAGE *IN VIVO* BY FDURD IN MICE BEARING COLON CARCINOMA #26 TUMORS

C. Schöber[1], S. Cao[2], M.B. Yin[2], H.K. Slocum[2], Y.M. Rustum[2]
[1]Hannover Medical University School, 30623 Hannover, Germany
[2]Roswell Park Cancer Center, Buffalo, NY 14263, U.S.A.

The relation between antitumor response and variation of DNA-damage of individual cells *in vivo* following FdUrd i.v.-bolus treatment was investigated in colon carcinoma 26 bearing BALB/c mice using the single cell gel alkaline electrophoresis (SCG-assay) according to SINGH *et al.* Ten mice received a single i.v. bolus of 1500 mg/kg bodyweight FdUrd, a group of 10 mice served as control. Immediately before and 24 h later, tumor cells were collected by fine needle aspiration and processed with the SCG-assay. In control animals, more than 90% of cells had damage with a migration pattern of less than 20 μm which was considered to represent little to moderate damage. In treated animals, about 60% of cells showed considerable DNA-damage in excess of 20 μm with only 40% of cells showing DNA-fragmentation comparable to controls. All tumors showed a regression in tumor size but only 4 out of 10 were partial remissions. The extent of regression correlated to the amount of bulk DNA ($P = 0.046$). The SCG assay is able to define subsets of tumor cells uneffected by the action of FdUrd treatment *in vivo*. The high proportion of undamaged calls in treated animals may explain the relative resistance of this tumor.

108 ORAL

ANTIPROLIFERATIVE ACTIVITY AND MODE OF ACTION OF NOVEL COMPOUNDS OF MARINE ORIGIN

G. Balconi[1], E. Erba[1], M. Bonfanti[1], S. Filippeschi[1], T. Garcia de Quesada[2], J.M. Jimeno[2], M. D'Incalci[1]
[1]Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy
[2]PHARMA MAR, Tres Cantos, Madrid, Spain

LoVo and mdr-1 overexpressing LoVo/DX cells were exposed for 1 or 24 h to 14 compounds with novel structures (mycaperoxide B, Kahalide, isobengazole, crambescidin, thiocoraline, isohomoalichondrin B, epidioximanadic acid A, sesbanimida, lamellarin, MB-2, LL-15, Palauamine, compound 21 and dehydrodidemnin). The cytotoxic potency ranged from <1 pM to >1 μM. Most drugs were less active against LoVo/DX than against LoVo cells with few exceptions (e.g. isohomoalichondrin B). Flow cytometry studies showed that some compounds caused an accumulation of cells in G_2M phases. Detailed studies on the mode of action of these drugs are in progress. Preliminary data indicate that thiocoraline inhibit the decatenation reactions catalyzed by DNA-topoisomerase II, suggesting that a novel topoisomerase II inhibitor has been identified.

109 ORAL

ECTEINASCIDIN (ET) 743: DEVELOPMENTAL STATUS OF A MARINE (M) DERIVED ANTICANCER COMPOUND (AC)

G. Faircloth, J. Avila[1], J.L. Fernández Puentes, K. Rinehart[2], M. García-Rocha[1], D. García Grávalos, T. García de Quesada, J. Jimeno
PharmaMar R+D, Boston–U.S.–Madrid/León–Spain
[1]CBM-UAM, Madrid, Spain
[2]CBM-UAM, U. Illinois, U.S.A.

ET743 belongs to a new class of MAC isolated from a Caribbean tunicate. ETs are isoquinolines related compounds sharing antiviral, immunosuppresive and antitumour activity (ANT). ET-743 has *in vitro* specificity in melanoma (MEL) and NSCLC cell lines ($IC50_s$ <10 nM); ET743 inhibits DNA, RNA and protein synthesis at ET[] 0.03, 0.008 and 0.1 ng/ml, respectively. ET743 has a novel effect on the organisation of the microtubule network in COS1 and HELA cells at 40 nM (being reversible and not competitive with colchicine). Moreover ET-743 binds