Symposium no. 5: Molecular Basis of Drug Resistance

5.007

SIMULTANEOUS DETERMINATION OF P-GLYCOPROTEIN (P-170) EXPRESSION AND DNA CONTENT BY MULTIPARAMETER FLOW CYTOMETRY (FCM). M. Danova[1], M. Giordano[1], V. Candiloro[2], G. Mazzini[3], S. Palmeri[3], G. Ucci[1], A. Riccardi[1]. [1]2nd Medical Clinic, [3]Centro Studio Istochimica CNR, Univ. and IRCCS San Matteo Pavia, and [2]Dept. Oncology Univ. Palermo, ITALY.

Nuclear DNA content has prognostic importance with regard to survival in cancer patients, moreover, the resistance of tumor cells to chemotherapy is a major obstacle to successful cancer treatment. We developed a method to quantitate, on a per cell basis, MDR gene expression (level of intensity and % of positive cells) and DNA content (tumor ploidy and proliferative activity) with high resolution bivariate FCM. We studied parental and adriamycin-resistant cells from the human colon carcinoma-derived cell line (LOVO-4) and the erithroleukemia cell line (MELC-3), tumor samples from 5 untreated patients with colon cancer and bone marrow plasma cells (BMPC) from 20 patients with multiple myeloma (MM) before and after treatment. Results show that: 1) in both LOVO and MELC adriamycin-resistant cells, the P-170 level was 3-5 fold higher than in the parental cells, without a correlation with a particular cell cycle phase; 2) heterogeneity in P-170 expression was observed in colon cancer samples (which showed diploid DNA content); 3) in MM, hyperdiploid BMPC had greater p170 positivity intensity than diploid BMPC and the % of P-170 positive cells cells served to distinguish sensitive from resistant disease. Supp. by: C.N.R. Target Project "Biotechnology and Bioinstrumentation", A.I.R.C. and I.R.C.C.S. San Matteo.

5.008

THE PHENOTYPE OF THE DRUG RESISTANT HEPATOCYTE

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In experimental hepatocarcinogenesis multiresistant liver cells appear and develop to carcinomas. The mechanisms of this acquired resistance is multifactorial. Drug activation is reduced, while drug detoxification is more efficient. Saturation of membrane lipids is reduced as is lipid peroxidation. Deregulation of the mevalonate pathway secure supplies of cholesterol and farnesyl, while increased amount of ubiquinone provide the cell with a potent antioxidant. Downregulation of cell surface receptors and signal transduction influence the response to growth regulatory influences. Increased transferrin receptors saturate growth promoting redox reactions and maintain intracellular pH and NAD.

The phenotype of the resistant hepatocyte is mimicked by many tumor cells resistant to anticancer drugs and radiation and is a useful model for studies of drug resistance.

5.009

31P NMR AND ENZYMATIC STUDIES ON METABOLIC ALTERATIONS ASSOCIATED WITH MULTI-DRUG RESISTANCE IN HUMAN LEUKEMIC T CELLS

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Purpose: to assess whether the MDR phenotype is associated with metabolic alterations at the level of enzymes devoted to cell detoxyfication, with particular attention to the pentose phosphate (PP) pathway.
Wild-type vinblastine (VBL)-sensitive human leukemic T cells (CEM 1.3) were cultured in the presence of sublethal doses of VBL, to produce the MDR variant cell line CEM VBL-100 (Cianfriglia et al. Int.J.Cancer,45,95,1990). Phenotypic and genotypic characteristics of the two cell lines were assessed. PP pathway activation was studied by monitoring by 31P NMR (161 MHz) the concentration of 2-deoxyglucose 6-phosphate (2-dG6P) in cell extracts after cell incubation (37°C) in the presence of 2-deoxyglucose (2-dG) (Ferretti et al.,in prep.). In both cell types 2-dG was phosphorylated by hexokinase into 2-dG6P, which was not further metabolized through the glycolytic pathway and therefore accumulated in the cell, giving rise to a 31P NMR doublet signal at 4.5 ppm. Accumulation of 2-dG6P was much lower in the resistant with respect to the sensitive cell line in the first 2-3 h of incubation. This result could in principle be explained by a) decreased hexokinase (HK) activity; b) enhanced activity of glucose 6-phosphate dehydrogenase (G6PDH) and/or subsequent enzymes in the PP pathway:lactonase and gluconate 6-phosphate dehydrogenase (6PGDH). Preliminary enzymatic studies indicated that the MDR phenotype was associated with A) unaltered HK and G6PDH activities; B)reduced activity of 6PGDH; C) higher levels of reduced glutathione. These findings suggest the interest of further investigating the role of cell detoxyfication mechanisms in MDR expression. (We thank AIRC and CNR PF BBS for financial support; Mr. M. Giannini for technical assistance).

5.010

P-GLYCOPROTEIN EXPRESSION IN RELATION TO NATURAL RESISTANCE IN UNTREATED HEAD AND NECK SOLID TUMOUS.

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Recently it has been proved that the natural or acquired resistence are associated to the presence of a membrane glycoprotein called P-glycoprotein, expressed in the multidrug resistent tumoral phenotype.
The authors have studied the overexpression of the glycoprotein on head and neck solid tumours ,using C129 monoclonal antibody by immunofluorescence.
Twentyfiveuntreated patients affected by head and neck tumours of which:15 larynx,6 nasopharynx and 4 tongue, have been taken in our consideration.
Contemporaneously the in vitro chemosensitivity to the vincristine and doxorubicin was evaluated by flow cytometry.The percentage of positive patients to P-GP has been of 20%, while the 32% showed an in vitro resistence to the VCR and DOX.These results show that head and neck tumours are abble to express mechanism of resistence to the antiblastics different from MDR.

5.011

HUMAN SERUM INHIBITS CYTOTOXIC ACTION OF SPERMINE-FCS ON K526 CELLS

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In our previous work the cytotoxic effect of spermine (100 µM) in the presence of 10% of FSC (in RPMI 1640 medium suplemented with L-glutamine and antibiotics) was clearly documented. The observed effect was specific for the FCS and was missing when 10% of human serum instead of FCS was present in the medium.

In this work we investigate the influence of some human sera (from group of healty people, and from group of patients with malignant melanoma) of the cytotoxicity of spermine-FCS. Results obtained indicate that human sera act inhibitory.

5.012

Potentiation of cholchicine action in sensitive and resistant B16 melanoma cells.

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Previous studies from our laboratory have shown a correlation between intracellular ATP content, cell growth and differentiation of B16 melanoma cells. In this study we examined the effects of novobiocin and alanosine on colchicine sensitive and resistant B16 melanoma cells. Novobiocin and alanosine, moderately decreased ATP levels in colchicine-sensitive cells. Both agents potentiated the anti proliferative effect of colchicine in these cells. Novobiocin was found to overcome cholchicine resistance in drug resistant cells. This effect correlated with ATP levels. The relationship of these findings to the multi-drug resistance trait is currently evaluated.