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Cytotoxic activity of doxorubicin and ETA 42 immunoconjugates against drug resistant small cell lung cancer cell lines *in vitro*

B Froesch, U Zangemeister-Wittke, K Rose and RA Stahel

Division of Oncology, Department of Medicine, University Hospital, Zurich 8044, Switzerland.

Small cell lung cancer (SCLC) is characterized by high initial response rates to chemotherapy. However, the disease recurs in most patients in a drug-resistant form resulting in a 2-year survival of only 5%. The present study evaluates the possibility of overcoming the resistance to doxorubicin (DOX) of two different multiple drug resistant (MDR) cell lines selected in DOX, and is based on the use of monoclonal antibodies (mAb) to deliver cytotoxic agents to the cells. Two approaches have been pursued. The first builds on an immunotoxin which exerts a different mode of action compared with conventional chemotherapeutic drugs and thus should not be afflicted by mechanisms of MDR. Conjugation of a truncated form of pseudomonas exotoxin A, which lacks the cell binding domain I (ETA 42) to the mouse mAb MOC 31 recognizing an epithelial glycoprotein, resulted in a highly selective and potent immunotoxin. As determined in [^{3}H]leucine incorporation assays, the cytotoxic activity against not resistant and resistant cell lines was demonstrated by IC_{50} values ranging between 2.7×10^{-11} and 5×10^{-11}. In limiting dilution clonogenic assays the killing capacity approximated 4 logs against all antigen positive cell lines tested. The rationale of the second approach builds on the cellular uptake of DOX by receptor mediated endocytosis which could help to bypass essential pathways responsible for MDR. Therefore, DOX was linked to mAbs using a covalent glutaraldehyde linkage or an acid labile hydrazon bond which allows liberation of the drug in the acidic environment of lysosomes. Because the cytotoxic potency of mAb drug conjugates depends on the internalization rate and the intracellular routing of the mAb antigen complex formed on the cell surface, three different mAbs have been evaluated for their potential to make potent DOX immunoconjugates. In addition to MOC-31, DOX was conjugated to the mAbs SWA11 and ABL, recognizing the leucocyte differentiation antigen CD24 and the Lewis Y carbohydrate, respectively. As shown in cell proliferation assays, all conjugates were selectively active against antigen positive cells. Their cytotoxic potencies against MDR cell lines are under investigation.

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The MDR1-mediated multidrug resistance is regulated by cell density and tumor volume in colon carcinomas

Dominic Fan, Pedro J Beltran, Mordechai Gutman, Yun-Fang Wang, Corazon D Bucana, Diane Bielenberg and Isaiah J Fidler

University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.

The MDR1 product P-glycoprotein (P-gp) is a major determinant for tumor cell resistance to certain anti-cancer drugs. In addition to the influence of organ environments, the relative expression of MDR1 is downregulated by high cell density in the CT26 murine colon cancer cells, as determined by flow cytometry (P-gp), and by Northern analysis and *in situ* hybridization (MDR1 mRNA). This phenomenon was not readily linked to changes in cell division ([^{14}C]-thymidine uptake), protein synthesis ([^{3}H]-leucine incorporation), or to nutrient deprivation (spent medium). Downregulation of P-gp was also observed in the larger s.c. tumors of the KM12C human colon carcinoma cells grown in nude mice. These findings may partially explain the polarized expressions of MDR1 in colorectal carcinomas and the thin clinical association of P-gp with large resectable tumors. The results from CT26 murine colon carcinoma cells are shown below:

Culture	Cell density	[^{14}C]-thymidine	Relative units	
time (h)	(cells/mm^2)	(cpm/10^3 cells)	P-gp	MDR1-mRNA
4	76	7	52	3+
8	76	17	51	not done
24	109	158	43	4+
48	187	157	17	3+
72	717	5	7	1+
96	1216	5	1	0

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DNA topoisomerases and drug resistance

BS Sørensen, K Christiansen, B Knudsen and O Westergaard

Department of Molecular Biology, Aarhus University, DK-8000 Århus C, Denmark.

DNA topoisomerases are a group of enzymes with the capability to change the topology of DNA. This activity is embodied in the ability of the enzymes to cleave DNA, followed by a rejoining of the broken ends. The importance of topoisomerases in clinical pharmacology has become increasingly evident, as the enzymes have been identified as the cellular targets for a number of clinically employed anti-cancer agents. Chemo-