demonstrated occasional expression of MDR1 gene products in melanoma cells. However, results are contradictory in terms of presence and functional activity of P-gp. This study investigated whether P-gp-mediated MDR might contribute to chemoresistance in malignant melanoma. Several detection methods were evaluated with regard to their sensitivity and specificity for determination of a non-chemotherapeutically induced (intrinsic) MDR. Thirty-three human melanoma cell lines established from primary and metastatic lesions were investigated for P-gp expression immunocytochemically (JSP1, MRK16, C219) and semiquantitatively by radioimmunoassay. MDR1-mRNA was analyzed by means of polymerase chain reaction (RT-PCR) as well as slot blot analysis. Functional activity of P-gp was investigated in daunomycin accumulation and cytotoxicity assays. Eleven (33%) melanoma cell lines show positive immunostaining for P-gp which correlates with results of radioimmunoassay. By the PCR-based methodology, 25 (76%) melanoma cell lines are found to express different levels of MDR1-mRNA. Data obtained by slot blot analyses correlate with immunostaining results. No relationship between daunomycin accumulation or cytotoxicity and P-gp expression can be demonstrated. However, verapamil significantly modifies both parameters in P-gp-expressing melanoma cells. Although MDR1 gene products are frequently expressed in malignant melanoma cell lines, P-gp mediated MDR appears to be not a decisive factor for the chemotherapy resistance observed in this malignancy. Nevertheless, the P-gp dependent potentiating effect of verapamil suggests MDR as an additional protective mechanism in malignant melanoma.

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Modulation of drug efflux and GST activity in resistant leukemic cells: possible mechanisms for reversing MDR in leukemia

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The contribution of different mechanisms to drug resistance status and possible modulations were investigated in drug-resistant leukemia cell lines. The K562 cl.6 daunorubicin-resistant myeloid cell line, isolated in the LHMC laboratory, displays cross-resistance to several anti-tumor agents, decreased drug accumulation and increased drug efflux, and high levels of MDR1 gene mRNA and P-glycoprotein (P-gp), as assessed by MTT, RT-PCR and flow cytometry respectively. Resistance to daunorubicin (DAU) was found to involve two mechanisms, one that decreased drug accumulation by the P-gp mechanism and another that altered the glutathione redox cycle. Both K562 cl.6/DAU and CEM/VLB_{100} T-cell leukemia resistant cells can be sensitized by introduction of cyclosporin A (CSA) and cremophor EL (CEL). Addition of CSA and CEL to the resistant cells led to a dose-dependent increase in DAU accumulation and retention, while no such effect by addition of CSA or CEL was observed in the sensitive cells. However, no complete reversal of MDR was observed even using toxic doses of CSA or CEL. Glutathione-S-transferase-3 levels were significantly elevated in K562 cl.6/DAU cells compared with the parental cells. In CEM/VLB_{100} cells which exhibited an MDR phenotype of cross-resistance, P-gp overexpression and increased drug efflux, Cu/Zn SOD activity was consistently increased by 38% when compared with the CCRF-CEM parental cells. In contrast, the activities of glutathione peroxidase and catalase were similar in both cell lines. The reactive oxygen species (ROS) was greatly decreased in the MDR cells when compared to their parental cells, as measured by electron spin resonance spectroscopy. Ethacrynic acid, an inhibitor of GST, could partially augment the chemosensitivity of DAU in K562 cl.6/DAU cells. The data suggested that MDR in resistant leukemic cells not only involves a P-gp mechanism, but also a GST and SOD protection mechanism against drug-mediated ROS cytotoxicity. While several mechanisms can exist in a clone of resistant leukemia cells, the P-gp effluxing mechanism may make a major contribution to the mechanism of resistance in K562 cl.6/DAU and CEM/VLB_{100} cells. CSA and CEL can overcome MDR phenotype *in vitro* by modulating drug transport kinetics through inhibition of effluxing function.

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Translocation between chromosome 7 and 16 in MDR HL60 cells

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HL60/AR cells, shown to overexpress the MRP but not the MDR1 product, are pharmacokinetically very similar to MDR cells that overexpress P-gp. They also share many properties with the independently isolated HL60/ADR cells, but are distinct from the latter in terms of drug distribution patterns and in terms of their karyotype which demonstrates a large homogeneously staining region (HSR) in the long arm of chromosome 7. To further define the chromosomal abnormalities in HL60/AR cells we used fluorescence *in situ* hybridization applying whole chromosome 7 and 16 painting probes to the metaphase chromosome spreads of HL60/AR cells. Chromosomes stained with chromosome 7 paint demonstrated a terminal deletion of a long arm of one of the chromosomes. Chromosome 16 paint demonstrated complete staining of one chromosome and partial staining of another. Double staining confirmed the translocation between the long arm of chromosome 7 and the short arm of chromosome 16. These results raise the possibility that the HSR on chromosome 7 is derived from chromosome 16. The above techniques can be readily applied to the study of leukemic blasts from patients with acute myelogenous leukemia especially in those with chromosomal abnormalities involving chromosomes 7 and or 16.