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Short communication

Expression of cell surface P-glycoprotein by an Adriamycin-resistant murine fibrosarcoma

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Summary. *Analysis of the cell membrane of Adriamycin (doxorubicin)-resistant UV-2237 ADM^R murine fibrosarcoma cells revealed a 170,000-dalton component that is not found in the drug-sensitive parent or revertant cells. Immunoblot (Western blot) analysis showed that this component is similar to the 170,000-dalton P-glycoprotein found on the surface of Chinese hamster ovary cells that exhibit multidrug resistance. Thus, multidrug resistance and P-glycoprotein expression apparently can occur in a wide variety of cells, including the metastatic murine solid tumor cell line described here.*

Introduction

Recently we isolated and characterized an Adriamycin (ADM)-resistant murine fibrosarcoma line designated UV-2237-ADM^R, which is cross-resistant to several unrelated drugs, including actinomycin D, amsacrine, mitomycin C, vinblastine, and vincristine [7]. Previous studies in other cell lines have shown that such pleiotropic drug resistance is associated with decreased net uptake of drug, presumably mediated by changes at the plasma membrane level. Summaries of these observations and discussions of possible mechanisms for decreased drug accumulation have been published by Baker and Ling [1] and by Biedler and Peterson [3]. Working with colchicine-resistant Chinese hamster ovary (CHO) cell mutants, Ling and his colleagues identified a molecular alteration in the plasma membrane that apparently correlates with membrane impermeability; a 170,000-dalton cell-surface glycoprotein (P-glycoprotein) is expressed that is not evident in the drug-sensitive parent cells [8, 11, 13]. The expression of high-molecular weight plasma membrane glycoproteins has been associated with multidrug resistance in other murine [5] and hamster [3, 6, 8] cell lines and in a vinblastine-resistant human leukemia cell line [2]. The present study was undertaken to determine whether a similar membrane alteration occurs in UV-2237-ADM^R cells, which exhibit reduced drug uptake [7].

Materials and methods

The UV-2237-ADM^R line was isolated by continuous exposure of the UV-2237 fibrosarcoma [10] to increasing amounts of ADM, up to 1 μg/ml, as described previously [7]. The UV-2237-revertant line was obtained by culturing the UV-2237-ADM^R line in the absence of ADM for 4 months. All tumor lines were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids and L-glutamine. The UV-2237-ADM^R line was maintained in this medium plus 1 μg/ml ADM and was subcultured into drug-free medium for 8–10 days before testing.

N-Trifluoracetyladriamycin-14-valerate (AD-32) was a gift from Dr M. Israel, Sidney Farber Cancer Institute, Boston, Mass; mitomycin C was purchased from Bristol Laboratories, Syracuse, NY. The other antitumor agents tested in the assays were provided by Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Relative levels of drug resistance were calculated by measurement of the efficiency of tumor-colony formation in the presence of varying concentrations of drug, as described previously [7].

Membrane vesicles from UV-2237-parent, UV-2237-ADM^R, and UV-2237-revertant tumor lines were purified by isopycnic centrifugation and sucrose gradients [13]. Membrane protein was assayed by the method of Bohlen et al. [4] and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with the modifications discussed previously [5, 9]. Western blotting was performed according to the method of Towbin et al. [15]. Rabbit antiserum, raised against plasma membranes isolated from colchicine-resistant CHO cells, was used to stain the Western blot, and ^{125}I-labeled protein A autoradiography was used to visualize antibody binding to protein bands [5, 9].

Results

Plating efficiency of the three tumor lines in the presence of varying concentrations of ADM is illustrated in Fig. 1. The UV-2237-ADM^R line was 140 times more resistant to ADM than the UV-2237 parent line. UV-2237-revertant cells were only five times more resistant than the parent cells (Fig. 1). The UV-2237-ADM^R line maintained resistance in vivo, whereas the UV-2237 parent line and the UV-2237-revertant line were both sensitive to ADM in vivo (data not shown).

The cross-resistance of the UV-2237-ADM^R line to a range of drugs of diverse and unrelated structure is shown in Table 1.

Fractionation of the membrane components from the tumor cell lines by SDS-PAGE is shown in Fig. 2. The

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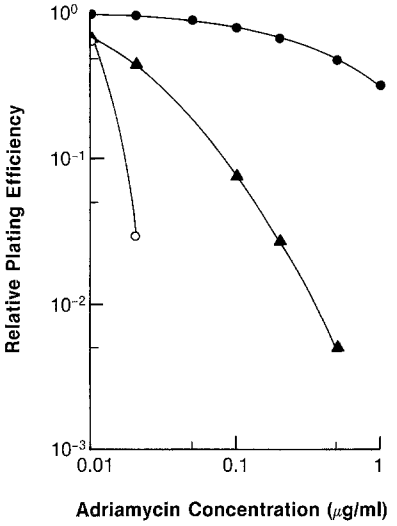

Fig. 1. Plating efficiency of UV-2237 cell lines in the presence of ADM. ●, UV-2237-ADM^R; ▲ UV-2237-revertant; ○, UV-2237 parent

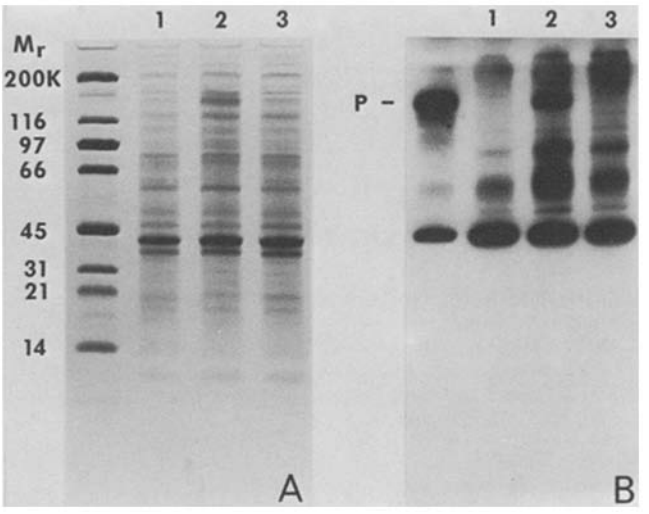

Fig. 2. *Panel A:* Plasma membranes were prepared from cells and fractionated by SDS-PAGE as described (see *Materials and methods*) Protein components were stained with Coomassie Brilliant Blue R250. *Far left lane* shows molecular weight standards from Bio-Rad containing myosin, β-galactosidase, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme. Lane 1, UV-2237 parent; lane 2, UV-2237-ADM^R; lane 3, UV-2237-revertant. One hundred and fifty microgram plasma membrane protein was loaded per lane. *Panel B:* SDS-PAGE was performed as in *panel A*, but gels were blotted onto nitrocellulose paper and probed with antibody against colchicine-resistant CHO cells [5, 9, 15]. *Far left lane* contains 20 μg CHO membrane from cells 1,000-fold resistant to colchicine. P-glycoprotein band is indicated *(P)* as identified previously [5, 9]. As in panel A: Lane 1, UV-2237 parent; lane 2, UV-2237-ADM^R; lane 3, UV-2237-revertant. Fifty microgram plasma membrane protein was loaded per lane

P-glycoprotein is visualized for the UV-2237-ADM^R line by protein staining, as shown in lane 2 of panel A. It appears as a heavily stained band migrating with an apparent molecular weight of about 170,000. Lanes 1 and 3 (Fig. 2, panel A), containing membrane preparations from UV-2237 parent and UV-2237-revertant cells, had much less evident bands at this position. The polyclonal antiserum recognized a number of other protein bands, but these did not show any consistent variation in different membrane preparations as the P-glycoprotein did.

Data obtained with the Western blot procedure confirmed these results, demonstrating that an antigen similar to the P-glycoprotein of CHO cells was expressed by the UV-2237-ADM^R line, but was undetectable in membranes from UV-2237 parent or UV-2237-revertant cells (Fig. 2, panel B).

Discussion

The results presented here demonstrate that UV-2237-ADM^R cells, which are resistant to ADM and cross-resistant to numerous drugs, express the P-glycoprotein at their cell surface. Expression of this 170,000-dalton glycoprotein appears to be associated with the drug-resistance phenotype as evidenced by the finding that neither the parent nor the revertant cells, both of which are drug-sensitive, expressed detectable amounts of this membrane component. The UV-2237-ADM^R cells thus resemble multidrug-resistant mutants isolated in the CHO cell system [8, 9, 11, 13].

Alterations in the physical properties of the plasma membrane, especially membrane fluidity, have been implicated in the mechanism of multidrug resistance [11, 12, 14, 16]. Altered mechanical and cell surface-related biological properties of drug-resistant cells have also been reported [2, 3, 13, 14, 16]. Although the membrane fluidity was not measured directly, we noted that the pressure required to disrupt the cell membrane of UV-2237-ADM^R cells was about half that required to disrupt the cell membranes of parent or revertant cell lines (data not shown). This observation is consistent with data previously reported for colchicine-resistant CHO cells [13].

Table 1. Cross resistance of the UV-2237-ADM^R line to common antineoplastic drugs

Drug	Index of relative resistance[a]	
	UV-2237 parent	UV-2237-ADM^R
Daunorubicin	1.0 (0.042)[b]	50.0
AD-32	1.0 (0.15)	8.3
Actinomycin D	1.0 (0.0002)	9.5
Amsacrine	1.0 (0.06)	11.6
Vinblastine	1.0 (0.0055)	44.0
Vincristine	1.0 (0.006)	109.0
Mitomycin C	1.0 (0.016)	26.0

[a] Level of resistance calculated by comparison of 10% plating efficiency of UV-2237-ADM^R cells in presence of drugs to that of UV-2237 parent cells

[b] Numbers in parenthesis are drug concentrations (μ*M*) that produce 10% relative plating efficiency

These observations suggest some interesting possibilities for the role of the P-glycoprotein in the ADM-resistant murine cells. The cross-reaction of a rabbit antiserum raised against hamster cells with the presumptive P-glycoprotein of mouse cells suggests that this protein is conserved, at least among rodent species. This in turn suggests that the P-glycoprotein is a functionally important molecule, although its specific role in multidrug resistance remains unknown. Our results and previous [5, 9, 13] data clearly indicate that P-glycoprotein is

associated with multidrug resistance. In addition, our study using the metastatic murine UV-2237-ADM^R cell line indicates that multidrug resistance and P-glycoprotein expression can arise in cells with a highly malignant phenotype and further supports the hypothesis that P-glycoprotein is ubiquitous in multidrug-resistant cells from different species.

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