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Short communication

Dominant expression of multidrug resistance in intraspecific murine lymphoma hybrid cells

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Abstract. Cultured P388/VCR mouse lymphoma cells resistant to vincristine (VCR) and to 5-bromodeoxyuridine (BUdR) and deficient in thymidine kinase (TK$^-$) were fused with P388/DAG cells resistant to 1,2:5,6-dianhydrogalactitol (DAG), an anticancer alkylating agent, and to 6-thioguanine (6-TG) and deficient in hypoxanthine phosphoribosyl-transferase (HPRT$^-$). The hybrid cells expressed multidrug resistance (MDR), i.e., resistance to VCR and cross-resistance to Adriamycin (ADM) and actinomycin D (Act. D), in a dominant manner. The presence of glycoprotein p170, the MDR gene product, was detected in the hybrid cells. Resistance to DAG was also expressed dominantly, whereas cross-resistance to dibromodulcitol (DBD), a chemically related anticancer drug, was slight.

Introduction

Multidrug resistance (MDR) and its circumvention has become an important field of theoretical and clinical research of cancer chemotherapy since Ling [1] published the pleiotropy of colchicine-resistant cells. Colchicine resistance was expressed in an incompletely dominant manner in intraspecific hybrid cells that were cross-resistant to vinblastine [2]. Further studies revealed that cells resistant to vincristine (VCR) showed cross-resistance to drugs structurally unrelated to the vinca alkaloids, e.g., Adriamycin, actinomycin D (Act. D) [3], and etoposide [4], among others.

It has been shown that cells expressing the MDR phenotype contain a cell-surface glycoprotein, p170, which is responsible for pleiotropic resistance [5]. According to the model for P-glycoprotein, it forms a channel in the plasma membrane and transports drugs out of cells using energy derived from adenosine triphosphate (ATP) hydrolysis. An alteration in the P-glycoprotein gene is the basis of the MDR phenotype [6]. In previous studies we showed that VCR resistance was expressed dominantly in P388 mouse lymphoma hybrids [7], which were cross-resistant to Adriamycin [8].

Hybridization has also been used to study resistance to alkylating agents [9, 10]. We observed an intermediate expression of resistance to the alkylating agent dianhydrogalactitol (DAG) in hybrids of sensitive and DAG-resistant P388 lymphoma cells [11]. DAG is not an MDR-type drug. It reacts with DNA by binding covalently to the guanine molecule, resulting in DNA interstrand cross-linking [12]. In DAG-resistant cells the resistance seems to be attributable to an increase in DNA repair [13].

In the present study we fused VCR-resistant and DAG-resistant mouse lymphoma cells to reveal whether MDR would be expressed dominantly and resistance to the alkylating agent DAG would be expressed intermediately in hybrids exhibiting both types of drug resistance.

Materials and methods

Cell culture. P388 mouse lymphoma [14] and its VCR-resistant subline (P388/VCR) were obtained from I. Wodinsky (Arthur D. Little Inc., Cambridge, Mass., USA). The P388/VCR tumor was first reported in 1965 [15]. DAG-resistant P388 cells were induced by giving increasing doses of DAG to P388-sensitive tumor-bearing mice [16]. Sensitive and resistant tumors were established in culture in Fischer medium containing 20% horse serum. Cells were growing in static suspension culture. The doubling times of P388/S (sensitive), P388/VCR, and P388/DAG cells were 12, 14, and 16 h, respectively. All three lines were capable of growing in semisolid agar medium, producing colonies within 8–12 days. The colony-forming ability was 60%–70%.

Cell fusion. The cell-fusion procedure used has been described elsewhere [11]. Briefly, enzyme-deficient sublines were produced by treating cells with ethyl-methanesulfonate (EMS) and plating them in either BUdR- or 6-thioguanine (6-TG) containing semisolid agar medium for the isolation of thymidine kinase (TK)-deficient P388/VCR-resistant and hypoxanthine phosphoribosyl transferase (HPRT)-deficient P388/DAG-resistant mutants, respectively. TK-deficient and HPRT-deficient cells were then fused with polyethylene glycol (PEG 6000, Fluka), and the hybrid cells were selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT medium).

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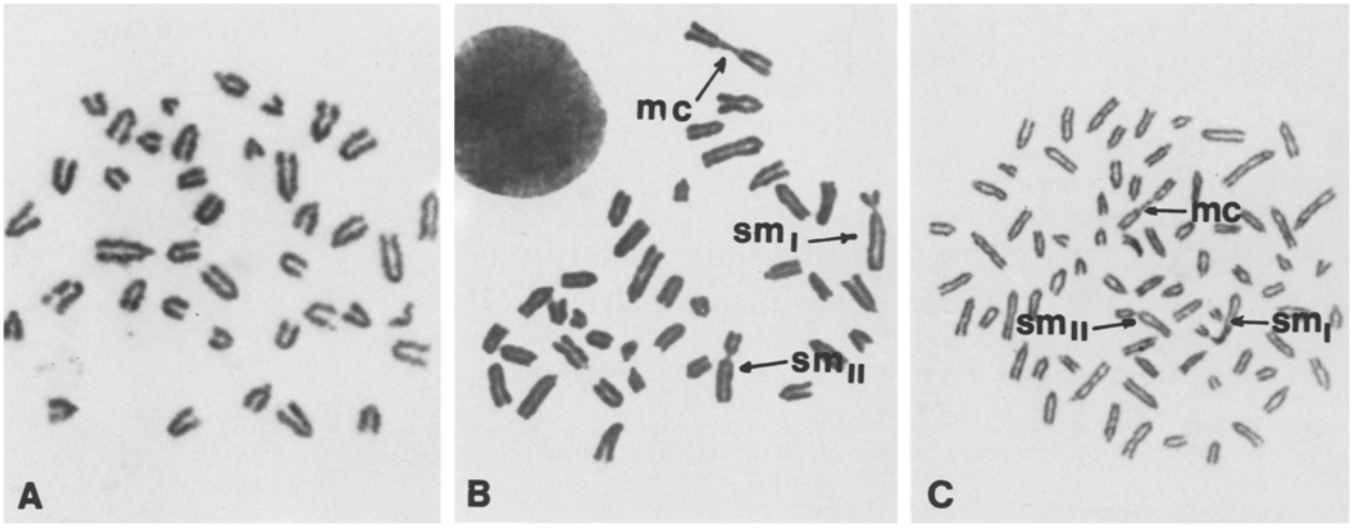

Fig. 1 A. Karyotype of a P388/VCR/TK-deficient cell. No metacentric marker is present. ×1630. **B** Karyotpye of a P388/DAG/HPRT-deficient cell. Two submetacentrics (sm_I, sm_{II}) and a metacentric marker with a long centromeric region (*mc*) are present (*arrows*). ×1630. **C** Karyotype of a hybrid cell. The cell is tetraploid and contains the three markers seen in **B** (*arrows*). ×1000 (Giemsa staining)

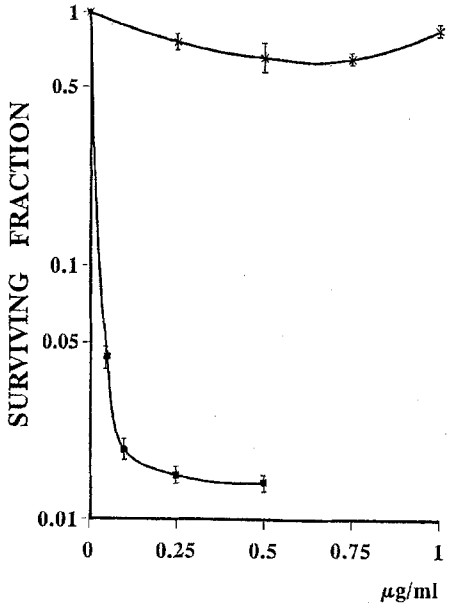

Fig. 2. Dose-response curves generated for P388 cell lines treated with VCR for 3 h. ■——■, P388/S; ✳——✳, P388/hybrid. *Vertical bars* represent the SEM

Chromosome spreads. Cells were treated with 0.1 μg colcemide/ml (Ciba) for 2 h and hypotonized in a 0.15% NaCl solution. The air-dried preparations were stained with Giemsa solution.

Dose-survival experiments. The drug sensitivity of the cells was determined by clonogenic assay in soft agar medium [17]. Briefly, sensitive P388/S cells and the P388/hybrid cells were treated with different doses of VCR for 3 h; with Adriamycin (ADM), Act. D, and DAG for 1 h; and with dibromodulcitol (DBD) for 2 h. Drugs were dissolved in physiological saline, with DBD being dissolved in dimethylsulfoxide and diluted with saline. Untreated control and drug-treated cells were plated in 50-mm plastic petri dishes in medium containing 0.25% agar and were incubated for 12 days in an incubator containing CO_2 (Heraeus). Dose-survival curves were drawn, from which IC_{50} and IC_{10} values were calculated as the drug concentrations needed to reduce the survival of treated cells to 50% and 10%, respectively, of the control values.

Detection of MDR: immunocytochemistry. Detection of p170 P-glycoprotein, responsible for the MDR phenotype, was carried out as described elsewhere [18]. Smears of cells on glass slides were fixed in acetone, rinsed, and placed in phosphate-buffered saline (PBS, pH 7.4). Primary anti-p170 antibodies C219 and 265/F4 [19, 20] were applied for 2 h. The slides were again washed and incubated with a biotinylated sheep anti-mouse second antibody (Amersham). After a washing step in PBS, the streptavidin-biotinylated-peroxidase complex (Amersham) method was carried out, and then a further wash was followed by developing 3-amino-9-ethylcarbazole/H_2O_2 for 5–10 min, which gives a red-brown reaction product in cells staining positively. Counterstaining was performed with hematoxylin.

Chemicals. VCR was supplied by Gedeon Richter Pharmaceutical Factory, Budapest. DAG and DBD were prepared by Chinoin Pharmaceutical Works, Budapest. EMS, 5-BUdR, 6-TG, thymidine, hypoxanthine, and aminopterin originated from Sigma. PEG 6000 was obtained from Fluka.

Results

Chromosome studies

The cytogenetic properties of the P388 lines, sublines, and mutants have been published previously [7, 11]. P388/S cells had one metacentric marker (M) chromosome, whereas P388/VCR cells lacked this marker (Fig. 1 A) [7]. P388/DAG cells had one metacentric marker with a long centromeric region (mc) and two submetacentric markers (sm_I, sm_{II}; Fig. 1 B) [11]. In the hybrid subline the modal chromosome number was about 80, the sum of that of the two parent cells (tetraploid value), and the cells contained all three markers (Fig. 1 C). These proved that a hybrid cell consisted of one VCR-resistant and one DAG-resistant cell.

Drug sensitivity

Hybrid cells of VCR- and DAG-resistant P388 sublines showed a high degree of resistance to VCR as compared

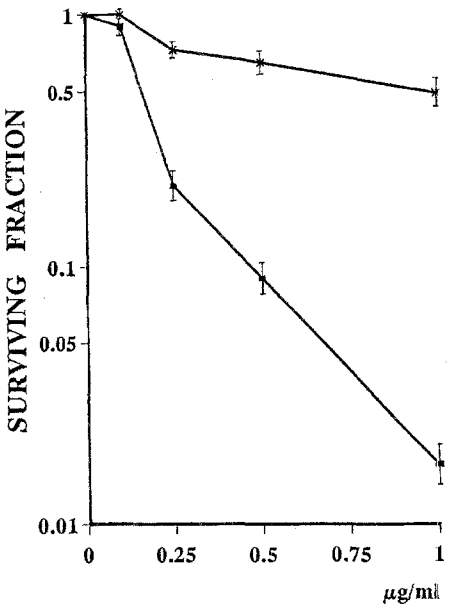

Fig. 3. Dose-response curves generated for P388 cell lines treated with ADM for 1 h. ■——■, P388/S; ✳——✳, P388/hybrid. *Vertical bars* represent the SEM

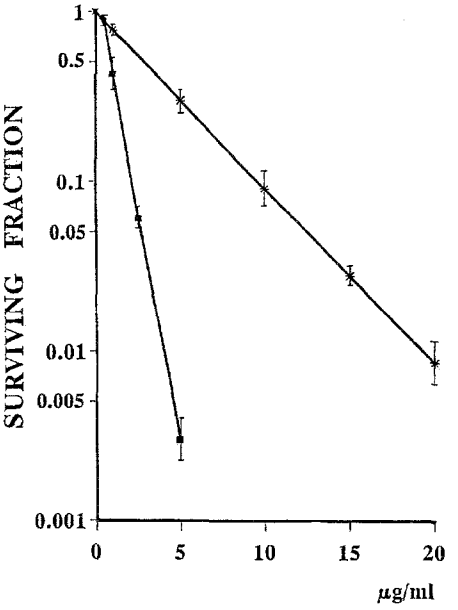

Fig. 5. Dose-response curves generated for P388 cell lines treated with DAG for 1 h. ■——■, P388/S; ✳——✳, P388/hybrid. *Vertical bars* represent the SEM

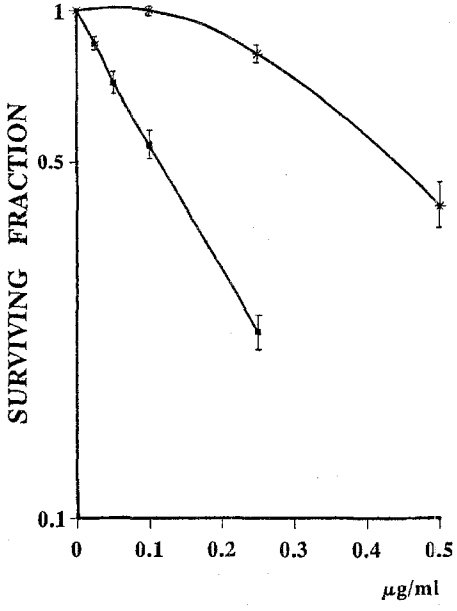

Fig. 4. Dose-response curves generated for P388 cell lines treated with Act. D for 1 h. ■——■, P388/S; ✳——✳, P388/hybrid. *Vertical bars* represent the SEM

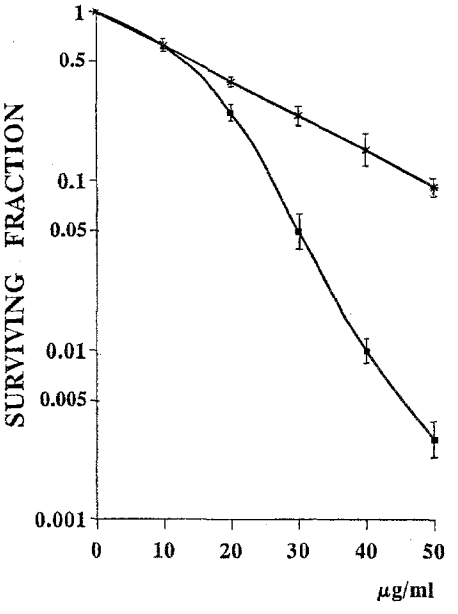

Fig. 6. Dose-response curves generated for P388 cell lines treated with DBD for 2 h. ■——■, P388/S; ✳——✳, P388/hybrid. *Vertical bars* represent the SEM

with the original parent line (Fig. 2). The difference amounted to a factor of more than 20. The second part of the curve (plateau) suggest the phase-specific character of the drug. A significant difference in sensitivity to ADM is shown in Fig. 3. P388/S cells were 6–10 times more sensitive than the hybrid cells. Similarly, Act. D was much more toxic to the sensitive P388/S parent line than to the hybrid line (Fig. 4). The difference, however, was less marked than that recorded for the other two drugs. The IC_{50} and IC_{10} values are shown in Table 1.

The dose-survival curves generated for DAG-treated cultures are shown in Fig. 5. Hybrid cells were shown to be more resistant to DAG than the P388/S cells. Treatment with DBD, another galactitol derivative with alkylating activity, showed a smaller difference in toxicity between the two lines (Fig. 6). The IC_{50} and IC_{10} values are summarized in Table 1.

Detection of the MDR gene product

The immunocytochemical reaction with the antibodies was practically negative in the P388/S cells. P388/VCR cells and the hybrid cells gave an intensive membrane-bound

Table 1. IC_{50} and IC_{10} values obtained in P388/S and hybrid cells

	VCR[a]		ADM[b]		Act. D[b]		DAG[b]		DBD[c]	
	IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}	IC_{10}
P388/S	< 0.05	0.04	0.15	0.48	0.11	0.19	0.75	2.0	12	25
Hybrid	> 1.0	> 1.0	0.98	> 5	0.43	0.6	3.0	9.8	15	48
Difference	>20×	>25×	6.5×	>10×	4×	3×	4×	4.9×	1.25×	2×
P388/VCR[d]	10×	>25×								
P388/DAG[d]							5.6×	5×		

Values are expressed in μg/ml
[a] 3-h exposure
[b] 1-h exposure
[c] 2-h exposure
[d] Resistance (*n*-fold) of resistant parental lines (earlier studies)

and cytoplasmic staining. The positivity of the reaction was strong (+++).

Discussion

Hybrid cells expressed both MDR and resistance to the alkylating agent DAG in a dominant manner. In previous experiments it has been reported that the MDR character is always inherited as a dominant trait either in hybrids from crosses of sensitive and resistant cells [2, 7, 8] or in hybrids produced from an MDR cell line (VCR, Act. D) and another type of resistant [methotrexate (MTX), cytosine arabinoside (ara-C)] cell line [21, 22]. The resistance of the hybrid cell to the other drugs (MTX, ara-C) was intermediate (MTX) or recessive (ara-C). When L1210 mouse leukemia cells showing the resistance to cyclophosphamide of the one partner and the resistance to methyl-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (methyl-CCNU) of the other partner were fused, resistance to both alkylating agents was expressed dominantly in the intraspecific hybrids [10].

It seems probable that the dominant expression of both types of resistance observed in the present experiments is attributable to the different mechanisms of resistance of the two drugs. In the case of DAG, the resistance of the P388/DAG cell is due to an increased repair of DNA [13]. Resistance to VCR is connected with the presence and activity of the membrane glycoprotein p170 [6]. We showed that the hybrid cell consisted of one VCR-resistant and one DAG-resistant P388 cell. P388/VCR cells had no metacentric marker (M) chromosome [7], whereas P388/DAG cells contained a metacentric marker with a long centromeric region (mc) and two submetacentrics (sm_I, sm_{II}) [11]. The hybrid cells were tetraploid, containing both sets of chromosomes and the three metacentrics. Only the true hybrid cells were capable of surviving in HAT medium. The dominance of MDR in hybrid cells was also shown by their strongly positive reaction with the monoclonal antibody. It should be emphasized that ploidy has no influence on drug sensitivity. Diploid and tetraploid P388/S cells were equally sensitive to drug effect [11].

In dose-response experiments the greatest difference in sensitivity between the sensitive and the hybrid cells was found in the case of VCR. The difference was less marked with ADM and was smallest with Act. D. This observation is in agreement with that of other investigators [23]. Hybrid cells were 4–5 times more resistant to DAG than the sensitive parental line. This finding is similar to the results reported for P388/SxP388/DAG-resistant hybrids [11]. Interestingly, the hybrid cells showed only minimal cross-resistance to DBD, in contrast to the results obtained previously [11]. The explanation for this discrepancy is that we used a short, 2-h exposure in the present study as opposed to "continuous" exposure in our previous study [11]. During the longer exposure period, DBD can transform to DAG by a solvolytic process in a slightly alkaline medium [24, 25], and the DAG newly formed from DBD contributed to the big difference found in our previous study. From the observations reported herein it can be established that intraspecific hybrids of MDR and alkylating-agent-resistant lymphoma cells express both resistant characters in a dominant manner. Hybrid cells were only slightly cross-resistant to DBD, an analog of DAG.

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