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The immunogenic properties of drug-resistant murine tumor cells do not correlate with expression of the MDR phenotype

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Abstract. Alterations in the immunogenic properties of tumor cells frequently accompany selection for multiple-drug-resistant (MDR) variants. Therefore, studies were performed to examine the hypothesis that overexpression of membrane P-glycoprotein, commonly observed in MDR tumor cells, is associated with enhanced immunogenic properties. Immunogenicity was determined by (a) the ability of drug-sensitive parental UV2237M fibrosarcoma cells and drug-resistant UV2237M variant cells to immunize normal mice against rechallenge with parental tumor cells and (b) the ability of normal syngeneic mice to reject cell inocula that caused progressive tumor growth in immunocompromised mice. Variant UV2237M cell lines included subpopulations selected for a six- to ten-fold increase in mRNA for P-glycoprotein and expression of the MDR phenotype (resistance to doxorubicin) and cells sensitive to doxorubicin (and no expression of MDR properties) but resistant to ouabain. All UV2237M drug-resistant cells were highly immunogenic in immunocompetent mice, regardless of their MDR phenotype. Additional studies showed that CT-26 murine adenocarcinoma cells, sensitive or resistant to doxorubicin (expressing high levels of P-glycoprotein), injected into normal syngeneic Balb/c mice produced rapidly growing tumors. The data do not demonstrate a correlation between the immunogenic properties of drug-resistant tumor cells and the expression of P-glycoprotein.

Key words: P-glycoprotein – MDR phenotype – Immunogenicity – Drug resistance

Introduction

The major cause of deaths from cancer is metastases that are resistant to systemic chemotherapy [3, 4, 9, 13, 23]. Resistance to anticancer drugs can be manifested at initial treatment or following repeated treatments [8, 17, 21] and arises through several cellular mechanisms. These include the selection of tumor cell subpopulations that possess altered permeability to drugs [1, 14, 30], heightened metabolic capacity to circumvent or repair damage induced by cytotoxic drugs [6, 7, 31, 38, 42], and the ability to redistribute drug throughout intracellular compartments and actively pump a drug from the cytoplasmic compartment into the extracellular space [5, 19]. This latter mechanism, drug efflux, is associated with the overexpression of the 170-kDa membrane-associated P-glycoprotein and results in pleiotropic drug resistance known as the multiple-drug-resistance (MDR) phenotype [21, 33, 36]. In addition, drug-resistant tumor cells frequently exhibit altered immunogenic and antigenic properties as compared to drug-sensitive, parental cells [24, 29, 32, 33]. Whether this relationship is associated with expression of P-glycoprotein is unknown.

Tumors that arise from chronic exposure of mice to ultraviolet light (UV) irradiation are usually highly antigenic and regress when transplanted into normal syngeneic hosts [26]. However, progressive variants of these tumors, such as the UV2237 fibrosarcoma, are able to grow and metastasize in normal mice [12, 28]. Variant UV2237 cell populations selected in vitro for resistance to doxorubicin (UV2237ADM^R and UV2237ADM^{RR}) possess the MDR phenotype [2, 20], overexpress P-glycoprotein as measured by relative levels of mRNA and cell-surface staining with C219 monoclonal antibody, and have served as models for studying in vivo mechanisms of drug resistance [10, 39]. During the course of these studies [39] we observed dramatic differences in the immunogenic properties of UV2237ADM^R and UV2237ADM^{RR} cells as compared to the parental population. It seemed reasonable that the antigenic topography of the drug-resistant tumor cells might be altered by the increased amount of P-glycoprotein

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within the plasma membrane, resulting in enhanced immunogenic properties. The purpose of the present studies was to determine whether the selection of cells with the MDR phenotype and enhanced expression of P-glycoprotein is invariably associated with altered immunogenicity in mice.

Materials and methods

Mice. Specific-pathogen-free, mammary-tumor-virus-negative C3H/HeN, Balb/c, and athymic (nude) mice were obtained from the Animal Production Area of the National Cancer Institute (Frederick, Md.). The mice were age-matched (6–12 weeks) within individual experiments. Animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current United State Department of Agriculture, Department of Health and Human Services, and NIH regulations and standards and used according to institutional guidelines.

Tumor cell culture. The UV2237 is a fibrosarcoma that was induced in a female C3H/HeN (mmtv$^-$) mouse by chronic UV irradiation [28]. The UV2237M subline is a metastatic line isolated in this laboratory. The UV2237ADM^R and UV2237ADM^{RR} cell lines were established by the selection of UV2237M cells to proliferate in the continuous presence of growth medium containing 1 μg/ml or 10 μg/ml doxorubicin respectively [20]. The UV2237^{rr} cell line was selected for its ability to proliferate in the continuous presence of 2 mM ouabain [3]. CT-26R100 and CT-26R500 are drug-resistant sublines of the CT-26 murine colon adenocarcinoma and were selected by growth in the continuous presence of doxorubicin. The designations R100 and R500 represent the fold increase in resistance to doxorubicin for 50% cell killing as compared to the parental cell line (IC_{50} = 2 ng/ml). All cell lines were grown as monolayer cultures in Eagles' minimal essential medium supplemented with 5% fetal bovine serum, vitamins, sodium pyruvate, L-glutamine, and nonessential amino acids. The complete medium was free of endotoxin as determined by the *Limulus* amebocyte lysate assay (Associates of Cape Cod, Mass.). Cultures were incubated at 37° C in a humidified atmosphere containing 5% CO_2 in air. All cultures were free of *Mycoplasma* and pathogenic murine viruses (assayed by M. A. Bioproducts, Walkersville, Md.).

Cytostasis assay. The antiproliferative effects of doxorubicin, vinblastine, and ouabain were determined by a sensitive colorimetric assay of viable cells [10]. Briefly, about 2000 cells were seeded into the wells of flat-bottom 96-well culture plates and allowed to adhere for 18 h. The cultures were then refed with either fresh medium or medium containing the indicated concentrations of drugs. After 5–6 days, the number of viable cells was determined by the addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 80 μg/ml for 1.5 h, followed by lysis of the cells and uniform solubilization of the dye with full-strength dimethylsulfoxide. The relative absorbance at 570 nm of individual wells was measured by a MicroFluor MR5000 microtiter plate reader (Dynatech, Alexandria, Va.). Cytostasis was calculated from the formula as,

$$\text{cytostasis } (\%) = (1-A/B) \times 100$$

where A is the absorbance of samples with drugs and B is the absorbance of samples with medium only. All experiments were performed at least twice. Student's t-test (paired) was used to determine statistical differences between samples at a confidence level of $P < 0.05$.

UV irradiation of mice. The light source was a bank of six FS40 sunlamps (National Biology Corp., Twinsburg, Ohio) that have a peak emission at 313 nm and deliver approximately 42% of their total energy within the UVA range (320–400 nm), 58% within the UVB range (280–320 nm), and less than 0.5% within the UVC range (below 280 nm). During UV irradiation, the mice were housed, five per cage, but individually separated on a shelf 20 cm below the fluorescent bulbs. The mice, which were unshaved, were irradiated for 30 min a session, three times a week for 12 weeks (259 kJ/m^2 total UV dose). This dose has been shown to be sufficient for the induction of systemic immunosuppression that allows for the growth of regressor UV-induced tumors [27].

DNA, RNA isolation, and hybridization for the murine mdr1 gene and mRNA. DNA was extracted from cultured tumor cells as previously described [34]. Briefly, the cell pellets were resuspended in 630 μl buffer (55 mM TRIS, pH 8.0, 110 mM EDTA and 110 mM NaCl). Proteinase K (0.5 mg/ml) and sodium dodecyl sulfate (SDS, 0.5% v/v) were added to the cells and incubated at 50° C for 8–18 h. The suspension was extracted once using phenol and several times using a phenol/chloroform solution and dialyzed against 50 mM TRIS, pH 8.0, 10 mM EDTA, and 10 mM NaCl overnight at 4° C. RNase A (70 μg/ml) was added for 1 h at 37° C. DNA digestions with the *Eco*RI restriction endonuclease were conducted at 37° C for 8 h, and Southern blot analysis/hybridization was performed as described [35, 40]. Nytran nylon blots (Schleicher and Schüll Inc., Keene, N. H.) were washed twice at 60° C with 15 mM NaCl, 1.5 mM sodium citrate, pH 7.2, and 0.1% SDS.

Total cellular RNA was extracted from cultured tumor cells using a guanidinium thiocyanate/hot phenol method as previously described [35]. For northern blot analyses, poly(A)-rich RNA was prepared by oligo(dT)-cellulose chromatography, fractionated on 1% denaturing formaldehyde/agarose gels, electrotransferred at 0.6 A to GeneScreen nylon membranes (Dupont Co., Boston, Mass.) and cross-linked with 120 000 μJ/cm^2 UV irradiation using a UV Stratalinker 1800 (Stratagene, La Jolla, Calif.). Hybridizations were performed as described [32]. Filters were washed three times at 65° C with 30 mM NaCl/3 mM sodium citrate, pH 7.2, and 0.1% SDS.

The DNA probes used in these analyses were: (a) a 4.3×10^3-base (4.3-kb) *Eco*RI restriction endonuclease DNA fragment from the plasmid λ DRII, containing the murine *mdr*1 cDNA [22] and (b) a 1.3-kb *Pst*I gene fragment corresponding to rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [15]. Each DNA fragment was purified by agarose gel electrophoresis, recovered using GeneClean (Bio 101, La Jolla, Calif.) and radiolabeled by the random-primer technique using ^{32}P-labeled deoxyribonucleotide triphosphates [11]. *mdr*1 mRNA expression was quantified on an LKB ultrascan IL laser densitometer (Pharmacia LKB Biotechnology, Uppsala, Sweden) by averaging three separate measurements in the linear range of the film using the left, middle, and right areas of each transcript band as well as the control GAPDH transcripts. Each sample measurement was then calculated as the ratio of the average areas between the 4.3-kb *mdr*1 transcript and the 1.3-kb GAPDH transcript.

Experimental design and tumor cell injections. Cultured tumor cells were given fresh medium 24 h before harvest. The cells were rinsed in Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (HBSS) and overlaid for 2 min with 0.25% trypsin/0.02% EDTA solution. The flask was tapped, and the cells pipetted to produce a single-cell suspension. The cells were then suspended in HBSS and their viability determined by trypan blue dye exclusion. Only cell suspensions with more than 90% viability were used for in vivo studies, and the cells were kept at 4° C during the period of time required for injection. The appropriate number of tumor cells was injected into the dorsal lateral flanks of mice in 0.1–0.2 ml. The immunogenicity of the cell lines was determined in two ways.

The ability of tumor cells to immunize normal mice against a subsequent lethal challenge of tumor cells was determined by the injection of tumor cells into the left hind limb of syngeneic mice and amputation of the tumorous limb when the tumor reached about 1.0 cm in diameter. The mice were then boosted with an injection of live tumor cells about 2 weeks after removal of the primary tumor and challenged 2 weeks later with either the cells used for the initial tumor injection or a different tumor. This design allowed us to establish the immunological cross-reactivity of the UV2237M and UV2237ADM^{RR} cells, as well as determine the ability of UV2237M cells to immunize against a subsequent challenge of UV2237M cells. In addition, most experiments measured the immunogenic properties of the tumor cell lines by the ability of normal mice to reject an inoculum of tumor cells that would cause tumor growth

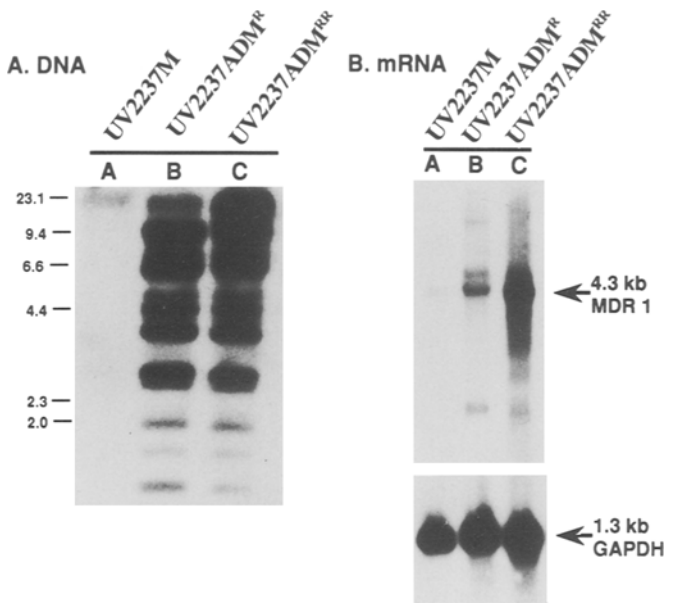

Fig. 1 A, B. Southern and northern blot analyses for *mdr*1 gene sequences and mRNA in UV2237M, UV2237ADM^R, and UV2237ADM^{RR} cells. **A** Southern blot analysis of chromosomal DNA isolated from the three cell types (*lanes A, B, C*). Hybridization with the murine 4.3×10^3-base (4.3-kb) *mdr*1-specific cDNA showed an increase in gene copy number in the drug-resistant cells. Ethidium bromide staining of the agarose gel before Southern transfer demonstrated equal loading of chromosomal DNA in each lane (data not shown). DNA fragment sizes were estimated by using *Hind*III-digested λ DNA as indicated on the *left* of the gel. **B** Northern blot analysis of *mdr*1 mRNA of the three cell types. Poly(A)-rich mRNA (5 μg/lane) was used in all cases. The probes used were 4.3-kb *Eco*-RI cDNA fragments of the murine *mdr*1 gene, which detects a 4.3-kb mRNA transcript [22]. *Lane A*, UV2237M; *B*, UV2237ADM^R; *C*, UV2237ADM^{RR}

in immunosuppressed animals. The data for tumor incidence were pooled from replicate experiments.

Results

The in vitro and in vivo properties of the UV2237M (parental) and the two drug-resistant sublines that possess the MDR phenotype, UV2237ADM^R and UV2237ADM^{RR} are summarized in Table 1. The cell lines had approximately equal doubling times in culture. UV2237ADM^R and UV2237ADM^{RR} cells exhibited about 1500- and 4000-fold increased resistance, respectively, to the cytotoxic effects of doxorubicin as compared to the parental UV2237M cells. The UV2237M cell line, although a progressor tumor in syngeneic C3H mice, is immunogenic as demonstrated by its ability to immunize against a lethal challenge of UV2237M cells following immunization with nondividing UV2237M cells [16] or resection of a primary tumor. In addition, the UV2237M and UV2237ADM^{RR} cell lines share common antigenic determinants with parental cells. We base this conclusion on the results showing that animals that either rejected an initial inoculum of 10^6 cells (about 50%) or had their primary tumors excised were immune to a lethal challenge of parental UV2237M cells.

Table 1. Properties of UV2237M, UV2237ADM^R, and UV2237ADM^{RR} cell lines

Characteristic	UV2237M	UV2237ADM^R	UV2237ADM^{RR}
In vitro doubling time (h)	12	14	15
Sensitivity to doxorubicin (IC_{50}, μg/ml)	0.01	15	>40
Ability to immunize against UV2237M cells	+	ND	+

The in vitro doubling time of cultured cells was estimated from the linear regression of growth curves. Cells were seeded at $(2-4) \times 10^3$ cells/well in 24-well plates and cell counts determined daily. The sensitivity of the cells to doxorubicin was estimated by MTT assay after growth of the cells for 5 days in the continuous presence of 0.001 – 50 μg/ml doxorubicin. The ability of the cells to immunize against the parental UV2237M cell line was determined by the injection of 10^5 cells (UV2237M) or 10^6 cells (UV2237ADM^{RR}) cells into the left hind footpad of C3H mice followed by amputation of the limb when the tumor reached about 1.0 cm. Mice were boosted with 10^5 viable tumor cells 3 weeks after resection of the tumor, and challenged with 10^5 UV2237M cells 1 week later, (see Materials and methods). ND, not determined

The overexpression of the murine *mdr*1 gene (P-glycoprotein) in the two drug-resistant cell lines was confirmed (Fig. 1). Figure 1 A shows the relative amounts of *mdr*1 DNA contained in the UV2237M, UV2237ADM^R and UV2237ADM^{RR} cells. Figure 1 B compares the amounts of the *mdr*1 4.3-kb mRNA transcript in these three cell lines. Expression of the 1.3-kb mRNA for GAPDH was measured as an internal control for the amounts of mRNA loaded in each lane. The relative amounts of *mdr*1 mRNA between the cell lines was quantified by calculating the ratios of the average areas between the 4.3-kb *mdr*1 transcript and the control 1.3-kb GAPDH transcript. When the UV2237M cells were normalized to a value of 1.0, the UV2237ADM^R cells contained six times more mRNA for *mdr*1 and the UV2237ADM^{RR} cells expressed ten times more *mdr*1 mRNA.

The immunogenic properties of these cell lines are described in Table 2. Whereas inocula of 10^5 or 10^6 parental UV2237M cells produced tumors in 100% of syngeneic mice, the drug-resistant sublines did not grow in immunocompetent mice at cell inocula below 10^6 cells (less than 50% incidence when mice are injected with 10^6 cells). However, the inoculation of as few as 12500 drug-resistant cells into nude mice resulted in 100% tumor incidence.

The data of Table 3 demonstrate that the highly immunogenic UV2237ADM^{RR} cells produced progressively growing tumors in mice that received chronic UV irradiation, a treatment known to induce immunosuppression that allows the growth of immunogenic UV-induced tumors [27]. An injection of either 10^5 UV2237M or 10^5 UV2237ADM^{RR} cells resulted in tumors in 10/10 and 8/10 UV-irradiated mice, respectively. Only the UV2237M cells caused tumors in normal, sham-irradiated mice. Nude mice injected with parental and UV2237ADM^{RR} cells served as positive controls for the ability of the cells to grow in vivo. Two of the subcutaneous tumors resulting

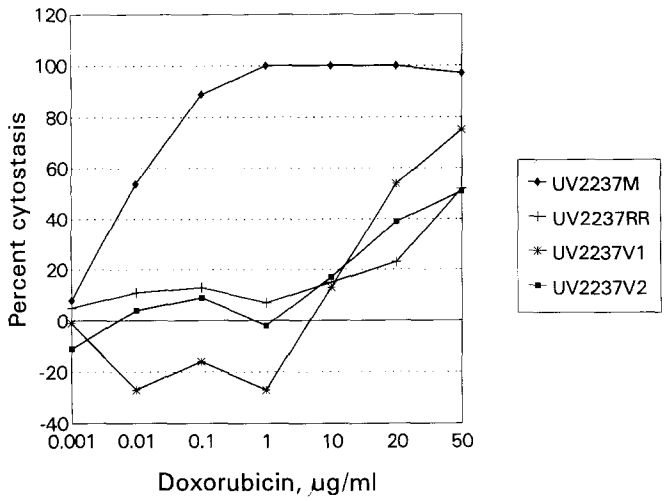

Fig. 2. Percentage cytostasis of UV2237M, UV2237ADM^{RR}, and two tumors derived in vitro from UV-irradiated mice inoculated with UV2237ADM^{RR} cells as a function of the concentration of doxorubicin. The excised tumors were passaged once in tissue culture and seeded into microtiter plates for assay of their sensitivity to the continuous presence of drug, as described in Materials and methods. UV2237M were the parental cells, UV2237RR the cultured UV2237ADM^{RR} cells, UV2237V1 and UV2237V2 the tumors derived in vivo

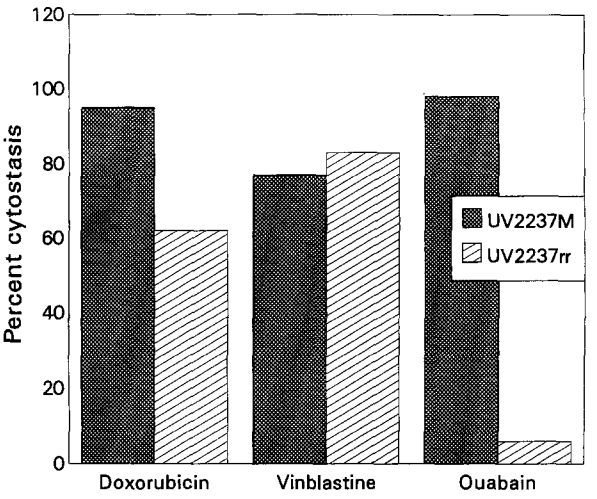

Fig. 3. Percentage cytostasis of parental UV2237M and ouabain-resistant UV2237^{rr} cells resulting from the continuous presence of doxorubicin (1 μg/ml), vinblastine (1 μg/ml), or ouabain (1 mM) in the culture medium. Viable cell number was determined by the MTT assay as described in Materials and methods. Values are the averages of replicate samples and the standard deviation was less than 10%. UV2237^{rr} cells showed no significant cytostasis to ouabain compared to the UV2237M cells (P <0.001)

from injection of UV2237ADM^{RR} cells in the UV-irradiated mice were excised, grown in cell culture, and tested for their drug resistance to doxorubicin. The results shown in Fig. 2 demonstrate that the tumor cells derived in vivo were almost 1000-fold more resistant to doxorubicin than were the cultured parental UV2237M cells.

To test further the hypothesis that the MDR phenotype correlated with enhanced immunogenicity, we examined the immunogenic properties of another variant subline of UV2237 cells, UV2237^{rr}, selected for its resistance to 2 mM ouabain [3]. As shown in Fig. 3, these cells did not exhibit an MDR phenotype: they were highly sensitive to the cytotoxic effects of either 1 μg/ml doxorubicin or vinblastine but were resistant to 1 mM ouabain (less than 5% cytostasis as compared with nearly 100% cytostasis for UV2237M cells). In addition, UV2237^{rr} cells did not overexpress P-glycoprotein (data not shown). The UV2237^{rr} cells were also highly immunogenic since they were tumorigenic in nude mice but not in immunocompetent mice (Table 4).

Table 2. Growth of UV2237M, UV2237ADM^{R} and UV2237ADM^{RR} cells in C3H and athymic (nude) mice[a]

Cell inoculum	C3H			Nude		
	UV2237M	UV2237ADM^{R}	UV2237ADM^{RR}	UV2237M	UV2237ADM^{R}	UV2237ADM^{RR}
10^6	10/10	ND	13/32*	ND	ND	5/5
10^5	10/10	0/10*	0/10*	5/5	5/5	5/5
5×10^4	10/15	0/10*	0/15*	10/10	5/5	10/10
2.5×10^4	7/15	0/10*	0/15*	9/9	5/5	10/10
12×10^3	1/5	0/10	0/5	5/5	5/5	5/5

[a] Results show no. tumors/no. mice injected. Cultured tumor cells were injected subcutaneously into the dorsal lateral flank as described in Materials and methods. Tumor growth was monitored for 90 days. ND, not determined

* P <0.001 compared to UV2237M tumor incidence by χ^2 analysis

Table 3. Growth of UV2237M and UV2237ADM^{RR} cells in UV-irradiated and sham-irradiated mice[a]

UV-irradiated mice		Sham-irradiated mice		Nude mice	
UV2237M	UV2237ADM^{RR}	UV2237M	UV2237ADM^{RR}	UV2237M	UV2237ADM^{RR}
10/10	8/10	9/9	0/10*	3/3	3/3

[a] Results show no. tumors/no. mice injected. C3H mice were irradiated as described in Materials and methods. The mice were injected with 10^5 cells on day zero and all mice were monitored for tumor growth for 90 days

* P <0.001 compared to tumor incidence for all tumors in UV-irradiated mice by χ^2 analysis

Table 4. Growth of UV2237^{rr} cells in C3H and nude mice[a]

Cell inoculum	C3H	Nude
10^6	0/10	5/5
5×10^5	0/10*	5/5
10^5	0/15*	10/10
5×10^4	0/5*	5/5
2.5×10^4	0/5*	5/5

[a] Results show no. tumors/no. mice injected. The indicated number of tumor cells were injected subcutaneously as described in Materials and methods. Tumor growth was monitored for 90 days

* $P < 0.01$ compared to tumor incidence in nude mice by χ^2 analysis

Table 5. Tumorigenicity of CT-26 and CT-26 drug-resistant murine tumor cells in syngeneic Balb/c mice[a]

Cell inoculum	CT-26	CT-26R100	CT-26R500
10^5	18/18	15/15	17/18
5×10^4	8/8	5/5	8/8
2.5×10^4	8/8	5/5	8/8

[a] Results show no. tumors/no. mice injected. Cultured tumors cells were injected subcutaneously into Balb/c mice in the lateral flank. Tumor growth was monitored for up to 90 days

A further test of whether the MDR phenotype correlated directly with the immunogenic properties of tumor cells was carried out by using the CT-26 murine colon carcinoma. As shown in Table 5, two sublines of CT-26, CT-26R100 and CT-26R500, caused tumors in normal, syngeneic Balb/c mice and nude mice at a low cell inoculum. These drug-resistant sublines also exhibit a 3- to 10-fold increase in the amount of mRNA for the *mdr*1 gene and a 20- to 50-fold increase in the cell-surface expression of P-glycoprotein (Z. Dong, unpublished data).

Discussion

Frequent associations between enhanced immunogenicity and resistance to anticancer drugs have been reported [24, 29, 32, 33] but the molecular nature of the tumor cell antigens that determine the immunological tumor/host relationship is unknown. The UV2237M tumor model described here afforded an opportunity to examine the potential role of an overexpressed, membrane-associated molecule, P-glycoprotein, in the immunogenic properties of a drug-resistant tumor.

The lack of correlation between the MDR phenotype and the immunogenic properties of tumor cells reported here is consistent with other studies demonstrating that drug-resistant cells exhibit either increased or decreased sensitivity to cytotoxic effector cells [18, 25, 37]. These earlier experiments, however, were limited to lytic events associated with effector cells and did not address the phenomena of host recognition of the immunogenic properties of the cells. Collectively, these data suggest that antigenic alterations of the tumor cell membrane that occur in cells that express the MDR phenotype are probably not a result of enhanced recognition of cell antigens in the context of increased numbers of P-glycoprotein molecules within the plasma membrane.

Fibrosarcomas that arise as a result of chronic UV irradiation are usually highly antigenic and only grow in mice in which T-cell-mediated immunity is suppressed [26, 27]. These antigenic tumors also grow in mice that receive sufficient UV irradiation to induce T-suppressor cells that are specific for antigens common to UV-induced tumors [27]. UV irradiation of normal mice was sufficient in the present study to allow growth of the highly immunogenic UV2237ADM^{RR} cells, suggesting that this form of immunosuppression could block recognition or effector events that normally would have resulted in this tumor being rejected by normal mice.

Progressor UV2237M cells still retain immunogenic properties, as defined by their ability to immunize normal mice against a subsequent lethal challenge of UV2237M cells [17], and cytotoxic lymphocytes that have the phenotypic markers of natural killer cells can be isolated from the peritoneal cavity of UV2237-immunized mice [41]. It is possible that overexpression of P-glycoprotein could enhance the immunogenicity of these inherently immunogenic cells. This possibility can be tested by transfection of the *mdr*1 gene into parental UV2237M cells. Such studies are in progress. It seems likely that UV2237M cells are able to grow in immunocompetent mice by one or more mechanisms that include the induction of T-suppressor cells [17, 27] and secretion of immunoregulatory molecules such as prostaglandins or cytokines, or that UV2237M cells may lack recognition molecules that lead to strong host immunity as manifested by specific cytolytic T cells. There is strong evidence for this latter mechanism [41]. Moreover, the UV2237ADM^{R} and UV2237ADM^{RR} cells are extremely sensitive to IL-2-dependent, lymphocyte-mediated cytotoxicity whereas the parental UV2237M cells are resistant (J. J. K., unpublished observations).

In summary, through the use of selected sublines of the UV2237 fibrosarcoma and the CT-26 adenocarcinoma, we observed the following combinations of biological properties: (a) MDR-associated regressor tumors (UV2237ADM^{R} and UV2237ADM^{RR}), (b) MDR-associated progressor tumors (CT-26R100 and CT-26R500), (c) drug-resistant, (non-MDR-associated) regressor tumor (UV2237^{rr}), and (d) drug-sensitive (non-MDR-associated) progressor tumors (UV2237M and CT-26). Thus, overexpression of the cell membrane molecule P-glycoprotein did not correlate with immunogenic properties in normal mice.

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