Anticancer Drug Sensitivity and Expression of Multidrug Resistance Markers in Early Passage Human Sarcomas

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ABSTRACT

We have established new human sarcoma lines and examined their sensitivity to common antitumor drugs and expression of putative multidrug resistance (MDR) proteins. Eighty-two sarcoma samples were transplanted in nude mice. Fourteen of these sarcomas were established as tumor cell lines. We determined a chemosensitivity profile to antitumor drugs (MDR drugs = doxorubicin, mitoxantrone, and vincristine; non-MDR drugs = cisplatin, ifosfamide, and bleomycin) for each tumor line *in vivo*. Response to chemotherapy with doxorubicin and ifosfamide was observed in 30–50% of these tumor lines. Our results obtained with xenotransplants are similar to the results documented in clinical trials in which doxorubicin and ifosfamide are effective in 30–50% of the patients. Furthermore, we examined expression of MDR-relevant markers like P-glycoprotein, MDR-associated protein, lung resistance protein, and mdr1 mRNA in these xenotransplants. A relationship between mdr1 mRNA expression and response to doxorubicin was demonstrated in >90% of our tumor lines. In six sarcomas with mdr1 mRNA expression, five were resistant against doxorubicin and cross-resistant against several other drugs, whereas from eight sarcomas, which lacked detectable mdr1 mRNA, seven were sensitive to doxorubicin and other drugs. We found lung resistance protein or MDR-associated protein expressed in three resistant and mdr1 mRNA-positive sarcomas. These results demonstrate that mdr1 mRNA expression is a putative marker for drug resistance in our sarcoma lines. We conclude, therefore, that inherent P-glycoprotein expression might be also responsible for drug resistance occurring in treatment of patients with sarcomas. The established tumor lines are useful for additional investigations on mechanisms of drug resistance in sarcomas and as models for preclinical screening of new antitumor drugs.

INTRODUCTION

A complex system of genetic, molecular biological, and biochemical mechanisms can cause resistance against different groups of cytotoxic drugs like anthracyclines, anthrachinones, podophyllotoxines, and *Vinca*-alkaloids (for an overview, see Refs. 1 and 2). This so-called MDR[2] is characterized by overexpression of membrane or cytosolic proteins that are encoded by the different genes. The product of the human *mdr1* gene (P-glycoprotein), which is reported to be responsible for resistance, acts like a drug-extruding pump reducing accumulation of cytotoxic agents inside malignant cells and thereby preventing their function (3, 4).

In the last few years, several resistant cell lines were selected after treatment with doxorubicin. However, some of these cell lines lacked detectable mdr1 mRNA and P-glycoprotein. Search for putative carrier molecules that might account for resistance led to the identification of MRP and LRP (5, 6). There is evidence that, like P-glycoprotein, MRP is related to an active outward drug transport mechanism (7). Drug-sensitive cells transfected with an expression vector containing MRP cDNA showed MRP overexpression and resistance to doxorubicin. In transfected sublines, the intracellular accumulation of doxorubicin is decreased and drug efflux is increased (8, 9). The *LRP* gene is located on chromosome 16, close to the genes coding MRP. The cytosolic-expressed LRP protein may mediate drug resistance, perhaps via intervention with transport processes (10).

Although it is clear that P-glycoprotein and the other proteins can cause MDR in cell culture, their role in clinical drug resistance remains controversial. In most clinical trials investigating MDR, the patients received a polychemotherapy with at least one non-MDR drug. A correlation of the drug resistance with the expression of MDR-relevant markers in these trials is problematic.

We, therefore, used a preclinical approach for identifying the relevance of specific drug resistance mechanisms. We investigated relevant protein and gene expression of drug resistance markers in xenografts and compared these results with the sensitivity to single-drug treatment. This model allowed direct correlation between resistance to a single drug and the expression of MDR markers. Additionally, a relation to the clinical course of disease was accomplished.

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[2] The abbreviations used are: MDR, multidrug resistance; MRP, MDR-associated protein; LRP, lung resistance protein; RT-PCR, reverse transcription-PCR.

MATERIALS AND METHODS

Animals. Male NMRI nu/nu mice from our breeding colony were kept under sterile conditions. Animals were provided with food (Sniff, Soest, Germany) and water *ad libitum* and were maintained on a 12-h light/dark cycle.

***In Vivo* Tumor Lines.** Sarcoma tissues were obtained from the Surgery Department of the Robert-Rössle-Klinik Berlin-Buch and characterized by experienced pathologists from the Pathology Department. Transplants (5 × 5 mm) were immediately implanted s.c. into nude mice. The width and length of the tumors were measured weekly with a caliper-like instrument, and tumor volume was calculated using the following formula: tumor volume = $(\text{width})^2 \times \text{length}/2$. When the tumors were ~12 × 12 mm in size, they were excised. A portion of the excised tumor was transplanted, a portion was frozen as stock, and a portion was prepared for histology. Histopathology studies and molecular assays confirmed the nature of the sarcomas.

Human α-Satellite-DNA PCR. DNA was prepared from tumor tissue using the QIAamp Tissue System (Qiagen GmbH, Hilden). Amplification of an 850-bp spanning centromer-specific fragment of human chromosome 17 was performed using modified primers corresponding to the primer pair 17α1/17α2 described by Warburton *et al.* (11). The 5′ primer covers the positions 15–39 and the 3′ primer covers the positions 867–891 of the sequence HSSATA17 (GenBank number M13882). For PCR, AmpliTaq-Gold polymerase and the corresponding PCR reagents from Perkin-Elmer Corp. were used (Applied Biosystems GmbH, Weiterstadt). Each reaction contained 200 μM dNTP, 250 nM of each primer, 2 mM $MgCl_2$, and 250 ng of genomic DNA. After an initial 10-min DNA denaturation and Taq activation at 94°C, 35 cycles with the following parameters were performed: 1 min at 94°C (denaturation) and 1 min at 60°C (annealing/extension), finally followed by a 10-min elongation at 72°C. PCR products were analyzed by agarose gel electrophoresis stained with ethidium bromide.

Chemosensitivity Profile. Groups of seven tumor-bearing animals each were randomized, either to receive antitumor drug treatment or to serve as control. When tumors were 5 × 5 mm in size, they were treated with following drugs and schedule: 10 mg/kg doxorubicin (i.v.; Farmitalia, Freiburg, Germany), 5 mg/kg mitoxantrone (i.v.; AWD, Dresden, Germany), 1 mg/kg vincristine (i.p.; Lilly, Bad Homburg, Germany), 8 mg/kg cisplatin (i.p.; Medac, Hamburg, Germany), and 400 mg/kg ifosfamide (Asta, Frankfurt, Germany) + mesna 200 mg/kg i.p. All treatments were of a single dose for 1 day, except for a 4-day treatment of 40 mg/kg body weight of bleomycin (i.p.; Mack, Illertissen, Germany).

Tumor volume and body weight were estimated weekly. Response of the tumor line was expressed as a percentage of the treated mean value against that of the control group (T/C). Significant sarcoma growth inhibition was confirmed by the nonparametric *U* test. After experiments were completed, all tumors were frozen for mdr1 mRNA analysis, and immunhistochemistry and cryopreserved with DMSO for a master stock.

Immunohistochemistry. To allow comparison of the expression of MDR markers with the results of chemotherapy, immunohistochemistry and RT-PCR were performed on tumor samples from the untreated control mice (between tumor passages 3 and 6). We used nonmalignant mesodermal tissues as control to determine basal expression of MDR markers in man.

We used a panel of three antibodies for determination of P-glycoprotein: the monoclonal antibodies JSB1 (Boehringer Mannheim, Mannheim, Germany) and C219 (CIS-Isotopentechnik, Dreieich, Germany) and the polyclonal mdr-AB-1 (Oncogene Science-Dianova, Hamburg, Germany). Frozen, fixed sections (5–8 μm) were incubated with appropriately diluted primary antibody in wet chambers for 1 h at room temperature. All sections were detected by using a PAP kit supplied by DAKO (Hamburg, Germany).

Immunohistochemistry for LRP, with monoclonal antibody LRP-56 and MRP with monoclonal antibody MRPm6 [both gifts from R. J. Scheper (Department of Pathology, Free University Hospital, Amsterdam, the Netherlands)] were done as described for P-glycoprotein.

RT-PCR. Analysis of mdr1 mRNA was performed by a method described earlier (12). Total cellular RNA was prepared by the method of Chomczynski and Sacchi (13). After isolation, RNA was examined by agarose gel electrophoresis for intact rRNA bands, and RNA concentration was estimated spectrophotometrically. Reverse transcription was carried out as follows: reaction tubes contained in a total volume of 10 μl, 1 μg of total cellular RNA, 5 pmol of one of the specific primers, 2 nmol each of dNTP, 10 units of AMV reverse transcriptase in 1 × reverse transcription buffer [50 mM Tris/HCl (pH 8.9), 75 mM KCl, 3 mM MgCl2, and 10 mM dithiotreitol], were allowed to proceed at 37°C for 1 h.

The cDNAs were amplified by PCR with the use of specific primers for the *mdr1* gene in humans and aldolase as a control gene. For PCR, 40 pmol of each primer either for mdr1 or aldolase, 10 nmol each of dNTP, and 1.5 units of Taq polymerase were added into the reverse transcription tubes in 1 × PCR buffer [10 mM Tris/HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, and 20 μg/ml gelatin] with a final volume of 50 μl. The PCR was performed for 45 cycles with the following parameters: 90 s denaturation at 94°C, followed by a 120 s annealing at 60°C and a 120 s extension at 72°C. PCR products (5 μl) were size-fractionated in a 2% agarose gel electrophoresis. Gels were stained with ethidium bromide and examined under UV light.

RESULTS

Characteristics of Sarcomas *in Vivo*. We implanted a total of 82 sarcomas of different subtypes s.c. into nude mice. Thirty-one tumors grew over two passages, 21 of these tumors grew over three passages, and at least 14 of them presented viable tumor tissue and tumor growth over more than three passages. The 14 regularly growing tumors were selected as tumor models with doubling times between 5 and 25 days. Histological and clinical data are shown in Tables 1 and 2. Eight of 14 sarcomas that were established as tumor lines originated from metastases or local recurrences.

With each passage in nude mice, a portion of the tumor tissue was fixed in formalin, and paraffin sections were prepared. After staining with hematoxylin, the tumor histology was compared with the original sarcoma structure. By transplantation of intact tissue, we maintained the integrity and characteristics of the original tumor. Hence, we found in nearly all tumor

Table 1 Characteristics of sarcoma lines

Sarcoma				Chemosensitivity T/C[a] (%) and expression of markers for MDR in xenotransplanted sarcomas											
				MDR drugs			Non-MDR drugs			P-glycoprotein, LRP, MRP,[b] and mdr1-mRNA					
No.	Subtype	TNM/Grading	Lesion	Do	Mi	Vi	If	Pt	Bl	C219	JSB1	mdr-AB1	LRP-56	MRP-m6	mdr1 mRNA
4013	Synovial sarcoma	$T_2N_0M_1$ G3	Lung metastasis	24[c]	79	96	11[c]	58	34	−	−	−	−	−	−
4126	Chondrosarcoma	$T_2N_0M_0$ G2	Primary	30	104	31[c]	145	88	61	++	+	++	++	++	+
4149	MFH	$T_2N_0M_0$ G2	Local relapse	169	107	99	85	37	133	++	++	++	+	+	+
4183	Rhabdomyosarcoma	$T_2N_0M_1$ G3	Primary	14[c]	33[c]	99	8[c]	76	29	−	−	−	−	−	−
4254	Malignant schwannoma	$T_2N_1M_1$ G3	Primary	133	53	84	288	117	48	−	−	−	−	−	−
4290	Chondrosarcoma	$T_2N_0M_1$ G3	Primary	66	29	62	26	69	90	ND	ND	−	ND	ND	+
4420	Malignant schwannoma	$T_2N_0M_0$ G2	Local relapse	39[c]	56	33	28[c]	107	38[c]	−	−	−	ND	ND	−
4448	Liposarcoma	$T_2N_0M_0$ G2	Local relapse	10[c]	21[c]	39	63	33	11[c]	−	−	−	−	−	−
4561	Synovial sarcoma	$T_2N_0M_0$ G3	Lung metastasis	13[c]	27[c]	65	66	172	17[c]	−	−	++	−	−	−
4605	Osteosarcoma	$T_2N_0M_0$ G3	Lung metastasis	21[c]	110	46	7[c]	15	12[c]	−	−	−	−	−	−
4618	Liposarcoma	$T_1N_0M_0$ G3	Local relapse	33	248	53	17	127	108	−	−	−	−	−	+
4679	Leiomyosarcoma	$T_2N_0M_0$ G3	Primary	169	84	88	70	53	100	+	+	+	+	−	+
4809	MFH	$T_2N_0M_0$ G3	Primary	17[c]	80	39[c]	42	33[c]	61	+	−	−	−	+	−
4917	MFH	$T_2N_0M_0$ G3	Local relapse	48	38[c]	17[c]	24[c]	58	20[c]	−	−	−	−	−	−

[a] T/C, tumor volume treated group/tumor volume control group × 100%; Do, doxorubicin; Mi, mitoxantrone; Vi, vincristine; If, ifosfamide; Pt, cisplatin; Bl, bleomycin; MFH, malignant fibrocytic histiocytoma; ND, not done.

[b] −, negative; +, 25% positive; ++, >50% positive cells.

[c] Significant reduction of tumor growth in treated group compared with the control.

Table 2 Comparison of sensitivity to chemotherapy in patient *versus* xenotransplant

	Xenotransplant (T/C)[a]		Patient	
Sarcoma	Doxorubicin	Ifosfamide	Chemotherapy with	Result
4013	29[b]	10[b]	Ifosfamide	P
4126	39	135	ND	
4149	88	68	Doxorubicin, ifosfamide	PR for 6 mos
4183	21[b]	7[b]	ND	
4254	90	163	Epirubicin, ifosfamide, vincristine	P
4290	62	33	Doxorubicin, ifosfamide	PR >4 mos
4420	39[b]	28[b]	Doxorubicin, ifosfamide	SD for 5 mos
4448	4[b]	45	Doxorubicin, ifosfamide	SD for 6 mos
4561	14[b]	85	Doxorubicin, ifosfamide	SD for 15 mos
4605	25[b]	7[b]	Cyclophosphamide, doxorubicin, vincristine, cisplatin	CR for 36 mos
4618	26	14	ND	
4679	169	70	Epirubicin, ifosfamide	CR >2 mos
4809	17[b]	42	ND	
4917	53	32[b]	ND	

[a] T/C, tumor volume treated group/tumor volume control group × 100%; CR, complete remission; PR, partial remission; SD, stable disease; P, progression; ND, not done; NE, not evaluable.

[b] Significant.

lines that the typical sarcoma morphology was present over at least six passages. An increase in tumor cellularity with higher passage was found in sarcoma lines 4126, 4290, 4448, and 4561, increasing polymorphy in line 4149 and round cell differentiation in line 4448.

As reported previously, for prostate tumors (14), not all xenografts retained a stable phenotype, and the explants were overgrown by transformed murine cells. Therefore, in addition to histological examinations, a PCR amplifying a 850-bp fragment of the α-satellite-DNA of the human chromosome 17 was performed on each xenograft to verify the human origin of the tumor material. As shown in Fig. 1, we found a strong positive signal for human α-satellite-DNA in each sample from our xenografted sarcomas, demonstrating that our xenografts retained a stable human phenotype during several passages in mice.

Chemosensitivity. We evaluated the response of the established tumor lines to drugs with known clinical efficacy against sarcomas. Drugs belonged either to the MDR group (doxorubicin, mitoxantrone, and vincristine) or were non-MDR compounds (cisplatin, ifosfamide, and bleomycin). The doses of drugs used were based on data from our laboratory and corresponded to the LD_{10}. Results of chemosensitivity tests on the sarcomas are summarized in Table 3. Additionally, in Table 1,

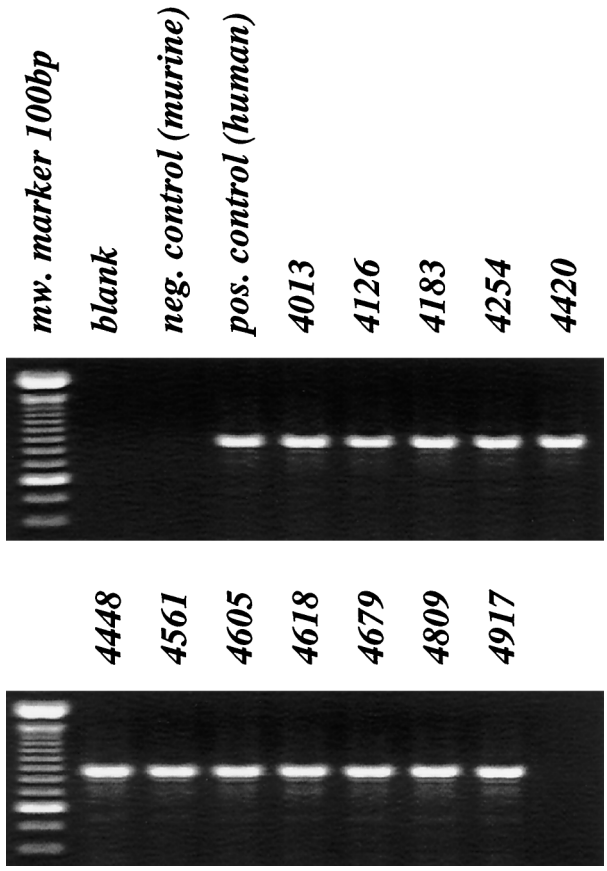

Fig. 1 Analysis of sarcoma xenografts for human DNA content. DNA was prepared from human sarcoma tissue, and an 850-bp spanning centromere-specific fragment of human chromosome 17 was amplified. PCR products were size-fractionated in a 2% agarose gel electrophoresis. Gels were stained with ethidium bromide and examined under UV light. *neg. control*, murine leukemia cells P388; *pos. control*, human breast carcinoma cells MV3366 + murine leukemia cells P388, 1+5.

the T/C (tumor volume of the treated group/tumor volume control group × 100) values were included. T/C values, based on the median tumor size during the experiments, allow an appropriate correlation of chemosensitivity with the expression of selected MDR markers. The established sarcoma lines showed a broad spectrum of chemosensitivity, ranging from good responses to complete resistance. Five of 14 sarcomas were resistant against all cytotoxic compounds used in the experiments. In 9 of 14 sarcomas, at least one drug was effective. In seven of these tumors, a treatment with doxorubicin led to a significant reduction of tumor growth. The tumor volume in the doxorubicin-treated groups was reduced by >60% in one sarcoma and by >75% in the other six sarcomas. Besides doxorubicin, also ifosfamide and bleomycin have been proved to be effective drugs for the treatment of sarcomas. With these drugs, we found a significant growth reduction in 5 of 14 sarcomas. Mitoxantrone was effective in 4 of 14 sarcomas, and vincristine was effective in 3 of 14 sarcomas. Cisplatin reduced tumor growth in only one line.

In some of our *in vivo* experiments, cross-resistance typical for MDR was not observed. For example, three sarcomas were resistant to vincristine and mitoxantrone, but sensitive to doxorubicin. In sarcoma 4126, vincristine inhibited tumor growth significantly, whereas doxorubicin and mitoxantrone were ineffective.

As shown in Table 2, 9 of 14 patients received chemotherapy after surgery. If we compare the tumor responses of the patients with results of *in vivo* testing of chemosensitivity in nude mice, we find a correlation in six of these sarcomas.

Immunohistochemistry. In preliminary investigations, using cryoslides from different normal mesoderm-originating tissues, we showed that nonmalignant cells do not express P-glycoprotein. Table 1 lists the results of immunohistochemistry staining of sarcoma specimens for P-glycoprotein using the three different antibodies. We found 5 of 13 tumors positive for P-glycoprotein. Three sarcomas were positive with all three antibodies, whereas the other two positive sarcomas were stained with only one antibody. We found that these three sarcomas that were P-glycoprotein positive with all three antibodies were resistant to doxorubicin and to most of the other drugs tested. Sarcomas 4561 and 4809 stained positive with only one antibody (sarcoma 4561 with mdr-AB1 and sarcoma 4809 with C219), and were both sensitive to doxorubicin and other drugs.

We investigated whether treatment with cytotoxic drugs induced a P-glycoprotein expression. Among the studied primarily P-glycoprotein-negative sarcomas (4183, 4290, 4420, 4448, 4561, 4605, 4618, and 4917), only one (4183) expressed P-glycoprotein after treatment with doxorubicin or mitoxantrone, as detected with the mdr-AB1 antibody.

Expression of the LRP was found in only three sarcomas. The expression of this protein seems to be associated with the expression of P-glycoprotein. We found MRP expression in three sarcomas (Table 1). Of these sarcomas, two were also P-glycoprotein positive.

mdr1 mRNA. In sarcomas 4126, 4149, 4290, 4448, 4618, and 4679, the results of RT-PCR analysis revealed expression of mdr1 mRNA (Fig. 2). Of these six sarcomas with mdr1 mRNA expression, four were resistant to doxorubicin and cross-resistant against all MDR drugs. Sarcoma 4126 was resistant against doxorubicin and mitoxantrone, but not cross-resistant to vincristine. In Table 1, we summarize the comparison between sensitivity of sarcomas to MDR-related drugs and expression of mdr1 mRNA. If we compare expression of P-glycoprotein and mdr1 mRNA in all distinctly P-glycoprotein-positive sarcomas (4126, 4149, and 4679), the results of immunhistochemistry are confirmed by mdr1 mRNA detected with PCR.

DISCUSSION

It was one of the most important problems in establishing the clinical relevance of MDR that most experiments have been done in cell culture. Such cell culture models with uniform cell lines are suitable for investigations of single mechanisms, but sometimes fail to address questions concerning the relevance of MDR in whole organisms. It has, however, been very difficult in clinical practice to correlate the response of drug treatment in patients with mdr1 mRNA and P-glycoprotein expression (15)

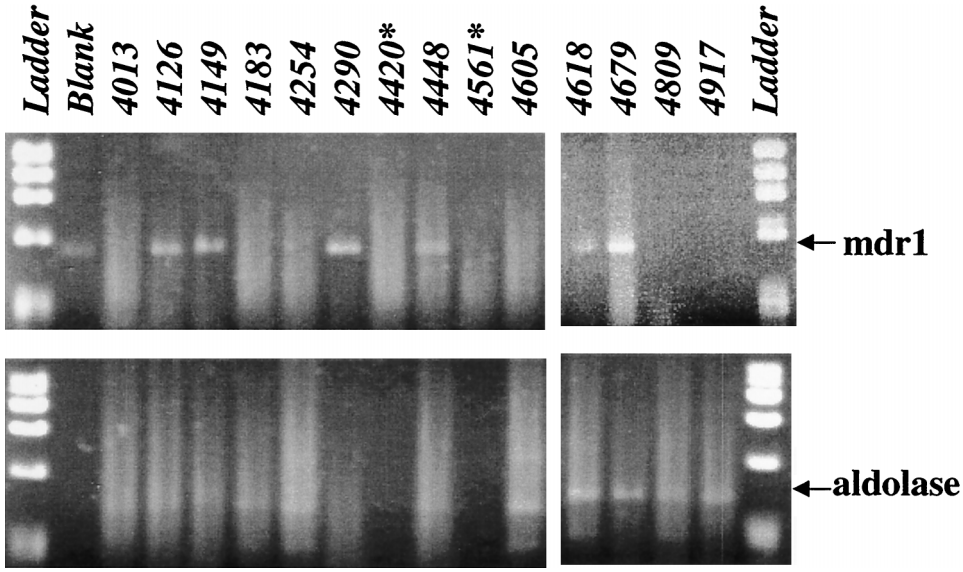

Fig. 2 RT-PCR analysis of mdr1 mRNA in human sarcoma lines. Total cellular RNA was prepared, and reverse transcription was carried out. The cDNAs were amplified by PCR with the use of specific primers for the *mdr1* gene in humans and aldolase as a reference gene. The PCR was performed for 45 cycles, and 5 μl of PCR products were size fractionated in a 2% agarose gel electrophoresis. Gels were stained with ethidium bromide and examined under UV light. ∗, PCR for sarcomas 4420 and 4561 did not work in this experiment; PCR was repeated and found negative for mdr1 and positive for aldolase.

because, in most cases, patients received combination therapy. Results of recent trials with MDR modifiers demonstrated that pharmacokinetic interactions were involved in drug resistance that could not be revealed in cell culture (16). Therefore, we decided to investigate the role of P-glycoprotein in drug resistance in mice. We used xenotransplants in nude mice to determine in parallel the expression of MDR-related proteins or genes and sensitivity to cytotoxic drugs. The drug-extruding function of these proteins are of special interest. We tried to investigate the function of P-glycoprotein by using the rhodamine 123 dye, as described earlier (17), but failed because most of our sarcomas did not grow in cell culture.

We transplanted 82 surgical sarcoma specimens into nude mice, and our initial take rate was 38%, comparable with other reports (18). Fourteen of these tumors were established as tumor lines and successfully passaged over 6 months. These established tumor lines showed a 100% take rate and had a tumor doubling time <25 days. By transplantation of intact large tumor pieces (4×4 mm^2), we found that the tumors preserved the typical structures of the original sarcomas. This was demonstrated in formalin-fixed sections in each passage. As reported previously for prostate tumors (14), not all human tumor xenografts retained a stable phenotype, and the explants were overgrown by transformed murine cells. Therefore, in addition to histological examinations, a PCR for a 850-bp fragment of the α-satellite-DNA of the human chromosome 17 was performed in each xenograft. By comparison with standards, we were able to validate the human background of the sarcomas growing in nude mice.

We determined a chemosensitivity profile for each tumor by treatment of these sarcomas with different drugs. Our results obtained in xenotransplants are similar to the results documented in clinical trials. Doxorubicin and ifosfamide seem to be effective in 30–50% of the patients. Another group investigating cytostatic drugs in sarcoma xenotransplants obtained different results (19). In their studies, doxorubicin was ineffective and only ifosfamide proved to be active. These experiments were done with tumor lines in higher passages and tumor doubling times <5 days. This might be one reason that doxorubicin was inactive in those models.

Comparison of our results obtained in xenotransplants with the clinical outcome in the patients (shown in Table 2) demonstrates a correlation between the two criteria within most tumors. In one patient (4013), however, the sarcoma was resistant against ifosfamide therapy, whereas the xenotransplant responded. This case may be an example for the development of resistance under clinical therapy because we investigated the chemosensitivity of this tumor before treatment with chemotherapy. Nonetheless, overall correlation between experimental and clinical data emphasize that our approach and tumor lines were an excellent basis for investigating drug resistance mechanisms in human sarcomas.

Our results support the hypothesis that expression of mdr1 mRNA and P-glycoprotein were involved in the drug resistance of sarcomas. In six sarcomas with mdr1 mRNA expression, five were resistant against doxorubicin and cross-resistant against other drugs, whereas seven of eight sarcomas, which were mdr1 mRNA negative, were sensitive to doxorubicin and other drugs. The overall correlation is $>90\%$. Immunohistochemistry was not completely able to reproduce the results obtained with PCR. There were three P-glycoprotein-positive sarcomas, and all of them were resistant; but in two other resistant sarcomas with positive mdr1 mRNA, no P-glycoprotein was detectable. The correlation, therefore, is $<60\%$. From our results, we recommend that both methods should be used in further trials or in clinical practice.

Many factors seem to influence the detection of P-glycoprotein in clinical specimens, including heterogeneous expression, immunological reagents with variable specificity, and different recognition epitopes. Thus, a group of investigators established guidelines (20) for the detection of P-glycoprotein and mdr1 in clinical specimens (St. Jude workshop). The recommendation to use three different antibodies, controls from well characterized mdr cell lines and nonmalignant tissue, and

Table 3 Sensitivity of xenotransplanted human sarcomas to chemotherapeutic drugs

Sarcoma	Day[a]		Control	Do[b]	Mi	Vi	If	Pt	Bl
4013		TV	0.428	0.122	0.324	0.437	0.042	0.259	0.169
	50	SE	0.083	0.038	0.069	0.097	0.004	0.063	0.063
4126[c]			0.138	0.053	0.135				
	49		0.042	0.023	0.050				
			0.539				0.725	0.304	
			0.220				0.249	0.042	
			0.082						0.063
			0.017						0.019
			0.176			0.079			
			0.035			0.019			
4149[c]			0.628	0.650	0.454				
	49		0.108	0.042	0.064				
			0.133			0.137	0.090	0.064	0.165
			0.034			0.051	0.011	0.026	0.065
4183[c]			0.852	0.183			0.056		
	49		0.141	0.068			0.009		
			1.277		0.368			1.250	
			0.259		0.036			0.219	
			0.715			0.549			0.312
			0.150			0.050			0.116
4254[c]			0.400	0.362			0.651		
	49		0.146	0.111			0.118		
			1.042		0.677	0.592		1.173	0.543
			0.306		0.135	0.134		0.155	0.068
4290			0.122	0.076	0.035	0.147	0.040	0.087	0.098
	49		0.031	0.011	0.007	0.044	0.011	0.011	0.022
4420			0.170	0.067	0.115	0.078	0.050	0.183	0.072
	50		0.031	0.008	0.021	0.019	0.013	0.030	0.008
4448			1.311	0.166	0.221	0.442	0.645	0.623	0.163
	28		0.416	0.024	0.037	0.073	0.053	0.174	0.014
4561			1.907	0.346	0.814	1.430	1.269	2.403	0.294
	46		0.343	0.157	0.341	0.366	0.285	0.327	0.091
4605			2.115	0.395	1.665	1.872	0.210	0.680	0.222
	33		0.596	0.135	0.782	0.666	0.091	0.324	0.057
4618			0.453	0.385	1.321	0.523	0.081	0.556	0.483
	48		0.098	0.177	0.349	0.167	0.010	0.183	0.088
4679			0.211	0.172	0.109	0.137	0.060	0.080	0.176
	50		0.079	0.039	0.023	0.041	0.016	0.014	0.046
4809			0.672	0.128	0.460	0.223	0.399	0.250	0.353
	48		0.124	0.038	0.103	0.060	0.136	0.048	0.081
4917			1.478	1.013	0.851	0.723	0.488	0.996	0.563
	19		0.230	0.123	0.103	0.209	0.065	0.150	0.118

[a] After start of treatment at which tumor volume was measured.
[b] Do, doxorubicin; Mi, mitoxantrone; Vi, vincristine; If, ifosfamide; Pt, cisplatin; Bl, bleomycin; TV, tumor volume (mean in cm^3).
[c] Sensitivity was determined in different experiments; each line represents one experiment.

freshly acquired tumor samples snap-frozen in liquid nitrogen was closely met by our study.

Expression of *mdr1* gene and P-glycoprotein in sarcomas was investigated previously (21, 22, 23, 24). Whereas Vergier *et al.* (23) found mdr1 mRNA in only 1 of 22 sarcomas, Stein *et al.* (24) showed that 42 of 61 sarcomas were mdr1 mRNA positive. Four human soft tissue sarcoma cell lines, established by Li *et al.* (25), were found to be P-glycoprotein negative and sensitive to doxorubicin. In all of these studies, a correlation with clinical response was absent. Levine *et al.* (26) examined the relationship of P-glycoprotein and mdr-1 mRNA expression with further parameters of clinical outcome in sarcoma patients. They found P-glycoprotein expression in 48% and mdr-1 mRNA in 51% of 65 sarcoma patients. The mdr-1 mRNA was not predictive for survival, whereas P-glycoprotein expression correlated with poor outcomes in sarcoma patients.

Our results, obtained in xenotransplanted sarcomas, are close to the results from Stein *et al.* (24), in which mdr1 mRNA was found in 60% of clinical sarcoma samples. It seems that our xenotransplanted sarcoma lines reflect closely the clinical situation.

It seems likely that the development of resistance is associated not only with P-glycoprotein expression but also other proteins. Among our tumor lines, there was at least one model in which the *mdr1* gene and P-glycoprotein were not involved in drug resistance. Therefore, we investigated MRP and LRP. We found that in this line (4254), also, both MRP and LRP were not involved in resistance. In the other sarcoma lines, we found MRP and LRP coexpressed with P-glycoprotein. If this coexpression occurs also in other tumors, this might be one reason for the insufficient activity of resistance modifiers mainly interacting with P-glycoprotein. Because most tumors in our study

being resistant to doxorubicin also revealed cross-resistance to other MDR-relevant and MDR-nonrelevant drugs, it would be difficult to propose a substitutional therapy in clinics after diagnosing resistance.

We conclude that P-glycoprotein expression plays an important role in drug resistance in human sarcomas. It might be possible to predict the results of chemotherapy by determination of P-glycoprotein and mdr1 mRNA and in this way to avoid unnecessary treatment. The established tumor lines are useful tools for additional investigations on mechanisms of drug resistance in sarcomas and can help to improve the knowledge about biology of sarcomas in patients.

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