Urol Res (1997) 25:35–42

ORIGINAL PAPER

M.J. Siegsmund · C. Kreukler · A. Steidler · T. Nebe
K.-U. Köhrmann · P. Alken

Multidrug resistance in androgen-independent growing rat prostate carcinoma cells is mediated by P-glycoprotein

Received: 13 February 1996 / Accepted: 26 June 1996

Abstract Prostate carcinomas are in general resistant against virtually all cytotoxic drugs. Up to now it has not been thoroughly evaluated whether specific resistance factors, such as the expression of the *MDR*1 gene, play a role in this multi-agent resistance and whether there is a link between drug resistance and hormone-independent growth. We investigated the resistance patterns of a hormone-sensitive and four hormone-independent Dunning rat carcinoma sublines against four drugs which are substrates of P-glycoprotein (vinblastine, taxol, doxorubicin, and etoposide) and two agents (methotrexate and *cis*-platinum) which are not transported by this efflux pump. All hormone-insensitive sublines, AT.1, AT. 3.1., MatLu and Mat LyLu, continuously showed a clearly enhanced resistance (3- to 26-fold) against the P-glycoprotein substrates, compared to the hormone-sensitive subline G. Only two of the androgen-independent sublines displayed enhanced resistance against methotrexate, whereas all of them were more sensitive against cisplatin than the androgen-sensitive G cells. By addition of verapamil the resistance against vinblastine (9- to 10-fold) and taxol (6.7- to 26.7-fold) in the hormone-insensitive cells could be almost totally reversed. Furthermore, the fluorescent P-glycoprotein substrate rhodamine-123 was effectively pumped out of the four tested hormone-independent cell lines, whereas the hormone-sensitive G cells were unable to extrude the dye. By reverse transcriptase polymerase chain reaction (RT-PCR) with primers specific for the rat *mdr1b* gene, the homologue to the human *MDR*1 gene, we could easily detect *mdr1b* expression in the androgen independent cell lines, but not in the G cells. Our results suggest that the product of the rat *mdr1b* gene is involved in the multidrug resistance of androgen-independent Dunning prostate carcinoma cells.

Key words Prostate cancer · Dunning tumor · Androgen-independent · Multidrug resistance · *mdr1b* gene

M.J. Siegsmund (✉) · C. Kreukler · A. Steidler
K.-U. Köhrmann · P. Alken
Department of Urology, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer, D-68135 Mannheim, Germany

T. Nebe
Institute of Clinical Chemistry, Faculty of Clinical Medicine Mannheim, University of Heidelberg, D-68135 Mannheim, Germany

Introduction

The standard treatment of metastatic prostate cancer is still androgen ablation, which results in a response rate of 70–80% with a duration of about 18 months. The use of chemotherapy in prostate cancer is limited because of two problems: (1) The patients that are affected with this cancer are usually old and have reduced organ reserves to withstand a therapy that is combined with multiple side effects [34]. (2) Prostate carcinomas are highly chemoresistant, which has been attributed to the fact that they grow very slowly, with a high number of cells resting in the G_0 phase of the cell cycle [35].

Specific resistance mechanisms that are actively expressed by cancer cells or mutations in target proteins of cytotoxic drugs have been demonstrated in a variety of different cancers. These include the expression of the membrane-bound drug transporters P-glycoprotein (Pgp) and multidrug-resistance-associated protein (MRP), detoxifying enzymes and peptides such as glutathione transferases, glutathione peroxidases, glutathione or metallothioneines, or mutations in topoisomerase genes [1, 8, 11, 15, 18, 26].

Pgp, the product of the *MDR*1 gene, is a member of the family of ATP-binding cassette transporters including other medically relevant genes responsible for cystic fibrosis and chloroquine resistance in *Plasmadium falciparum malariae*. The 170-kDa Pgp acts as a ATP-driven efflux pump that prevents cytotoxic drugs, such as vinca alkaloids, anthracyclines, and epipodophyllotoxins or

other natural product drugs, from accumulating intracellularly. Pgp and *MDR*1 mRNA have been detected in a variety of human tumors such as colon carcinoma, renal cell carcinoma, soft tissue sarcoma, acute myelogenous leukemia, multiple myeloma, non-Hodgkin's lymphoma, and others. *MDR*1 mRNA levels are frequently increased in chemoresponsive tumors that have become chemoresistant, suggesting a correlation of expression of *MDR*1 mRNA with acquired cytotoxic drug resistance [12]. A number of substances have been defined which interfere with the pump function of Pgp (i.e., verapamil, cyclosporin A, quinidine) and are therefore able to reverse multidrug resistance in vitro [21, 25, 30, 31] and in vivo [20, 23, 29]. Verapamil is the prototype for this group of agents and a powerful reverser of Pgp-mediated multidrug resistance in vitro. Its use in clinically resistant cancers would be tempting if it did not cause intolerable side effects. The role of Pgp in drug resistance of prostate cancers has not yet been defined. It is expressed in androgen-independent growing PC3 and DU145 prostate carcinoma cells [27]. Two publications describe a low or absent expression of *MDR*1 mRNA in a very limited number of prostate carcinoma specimens by using Northern and slot blotting [9, 10]. On the other hand, we were able to demonstrate low but probably clinically relevant levels of *MDR*1 mRNA expression in a high percentage of prostate cancers by reverse transcriptase polymerase chain reaction (RT-PCR) [24].

In this study we used different sublines of the Dunning rat prostate carcinoma to characterize their resistance profile and to define the role of the multidrug-resistance gene. We found a positive correlation between androgen-independent growth, the expression of the *mdr1b* gene, and the multidrug resistance phenotype.

Material and methods

Cell lines

The Dunning rat prostate carcinoma sublines G, AT.1, AT.2.1, MatLu and MatLyLu, which were a generous gift from Dr. John Isaacs,Johns Hopkins University, Baltimore, MD,USA, were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, L-glutamine (Gibco), penicillin (50 mg/ml) (Gibco), and streptomycin (50 mg/ml) (Gibco). The cell lines KB-3–1, KB-8–5, and KB-V1 were obtained from Dr. Michael Gottesman, National Cancer Institute, Bethesda, MD, USA. The drug-sensitive parental cell line KB-3–1 was derived from a single clone of human KB carcinoma cells after two subclonings. The vinblastine-resistant subline KB-V1 was obtained from the KB-3–1 cells after treating them twice with EMS (Ethylmethane Sulfonate), selecting the most drug resistant subclones, and subsequently increasing the vinblastine (Sigma) concentrations from 3 ng/ml to 1 mg/ml in mass populations. The colchicine-resistant KB-8–5 cell line was obtained by the same cloning and selection procedure, but the selection was stopped at a drug concentration of 10 ng/ml colchicine (Sigma), and the cells were further maintained in this colchicine concentration. The cell lines were grown in monolayer cultures at 37 °C in 5% CO_2 using Dulbecco's modified Eagle's medium with 4.5 g glucose/l, supplemented with 10% fetal bovine serum (Whittaker, MA Bioproducts), L-glutamine (Gibco), penicillin (50 mg/ml) (Gibco), and streptomycin (50 mg/ml) (Gibco).

1-(4,4-Dimethylthiazol-l-yl)-2,5-diphenyl Tetrazolium Bromid (MTT) Cytotoxicity assay

After trypsinization of subconfluent cultures, the viable, trypan blue excluding cells were counted in a hemocytometer.

The outer wells of a 96-well microtiter plate were filled with 100 ml of the appropriate medium without cells, creating a frame of liquid to prevent medium evaporation in the test wells. A quantity of 2000 cells/well were then seeded in rows 2–10 in a volume of 100 ml medium.The wells of row 11 were again filled with medium only, serving as blank. The cells were allowed to adhere to the bottom for 1 day before the appropriate drugs were added in falling concentrations from 1000 to 0 ng/ml in 100 ml medium. In chemosensitizing experiments, verapamil was used in a concentration of 5 µg/ml. At day 5, 20 ml 1-(4,4-Dimethylthiazol-l-yl)-2,5-diphenyl Tetrazolium Bromid (MTT) was added to each well, which was converted in the mitochondria of viable cells to a water-insoluble purple formazan precipitate. After 4 h of incubation with MTT, the medium was removed with a 20-gauge needle, fitted to a vacuum hose, the precipitate solubilized in 100 ml dimethylsulfoxide (DMSO), and the extinction read on an ELISA photometer at 540 nm (Titertek) The means and standard deviations were calculated of the six wells in each row and converted to percentage viable cells of the untreated controls.

The IC_{50} is the concentration of a drug that inhibits cell growth by 50%. The resistance-modifying factor (RMF) of verapamil was calculated as the quotient between the IC_{50} of a certain drug without verapamil divided by the IC_{50} of the combination of the same drug with 5 µg/ml verapamil.

Efflux of rhodamine-123

KB-3–1 and Kb-8–5 cells as well as the prostate carcinoma cells were preincubated at 37 °C in medium containing 1 mg/ml rhodamine-123 for 1 h. After a brief centrifugation, cells were resupended in medium without rhodamine-123. Aliquots were taken after 0, 15, 30, 60, and 120 min, diluted in ice-cold phosphate-buffered saline solution (PBS), washed twice, and resuspended in 400 ml cold PBS for fluorescence-activated cell sorter (FACS) analysis.

RNA extraction from prostate carcinoma tissues and RT-PCR

Total RNA was extracted from the tissues and cell lines using the acid-phenol method [6] and 1 mg was reverse transcribed in a reaction mixture consisting of 50 mM TRIS-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 1 mM dNTPs, 1 mg bovine serum albumin (BSA), 10 U RNAsin (Promega), 200 pmol random primers (BRL), 200 U Mo/MLV reverse transcriptase (BRL), and diethylpyrocarbonate (DEPC)-treated water in a final volume of 20 µl. The reaction mixture was incubated at 37 °C for 1 h and then used in the PCR reaction. The PCR reaction mixture consisted of 50 mM TRIS-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, (Deoxynucleotide triphosphates) and primers selected from the published cDNA of the rat *mdr1b* gene in a final volume of 100 µl. We used a hot-start PCR technique, where all reagents but the *Taq* polymerase were incubated at 94 °C for 5 min to denature all double-stranded DNA and prevent mispriming. At the end of this incubation period, 2.5 U *Taq* polymerase was added to each tube and a 30-cycle PCR started at 94 °C for 1 min, 52 °C for 2 min and 72 °C for 3 min with a final extension at 72 °C for 10 min on a Perkin Elmer thermocycler. The primers for the *mdr1b* gene were 5′-CGTCACCATGGATGAGATAG-3′ and 5′-GTCACTTTGGGATTCATAAG-3′, which produced a 459-bp fragment.

Results

Differential cytotoxicity of different cyotoxic drugs in androgen-sensitive and androgen-independent prostate carcinoma cells

To test for differential drug resistance in androgen-sensitive G cells and androgen-independent AT.1, AT.3.1, MatLu, and MatLyLu cells, we incubated them with increasing dosages of the cytotoxic drugs vinblastine, doxorubicin, taxol, etoposide (VP-16), cisplatin, and methotrexate (Table 1).

Vinblastine

Vinblastine, a known P-glycoprotein substrate, was the most effective drug in all tested cell lines, with IC_{50} values between 3.8 and 17.5 ng/ml. The androgen-independent growing cell lines AT.1, AT.3.1., MatLu, and MatLyLu were between 2.5 and 4.6 times more resistant against vinblastine than the androgen-sensitive G cells (Fig. 1).

Doxorubicine

Doxorubicine, also a P-gylcoprotein substrate, was overall less effective. The IC_{50} values ranged between 23 ng/ml for the G cells and 480 ng/ml for the AT.3.1 cells. With this drug the difference in resistance between the G cells and the androgen-independent cells was the highest, with IC_{50} values between 9.8-fold and 20.9-fold higher in the androgen-independent growing cells than in the androgen-sensitive cells.

Taxol

The novel drug taxol is also a substrate of P-glycoprotein. Overall, taxol was about as effective as doxorubicin, with IC_{50} values between 50 ng/ml in the G cells and 325 ng/ml in the androgen-independent MatLyLu cells. There also was a clear-cut difference in the response to taxol between the G cells (IC_{50} 50 ng/ml) and the androgen-independent growing cells, which were 4.8- to 6.5-fold more resistant to this drug.

Table 1 IC_{50} values of different cytotoxic drugs in five sublines of the Dunning rat prostate carcinoma (G, AT.1, AT. 3.1, MatLu, MatLyLu) (*Vbl* vinblastine, *dox* doxorubicin, *metho* methotrexate, *CDDP* cisplatin)

	Vbl	Dox	Taxol	VP-16	Metho	CDDP
G	3.8	23	50	172	9.6	1550
AT.1	15	233	240	700	32	165
AT.3.1	9.6	480	280	925	3.5	980
MatLu	17.5	225	320	975	25	610
MatLyLu	9.9	256	325	780	8.8	1320

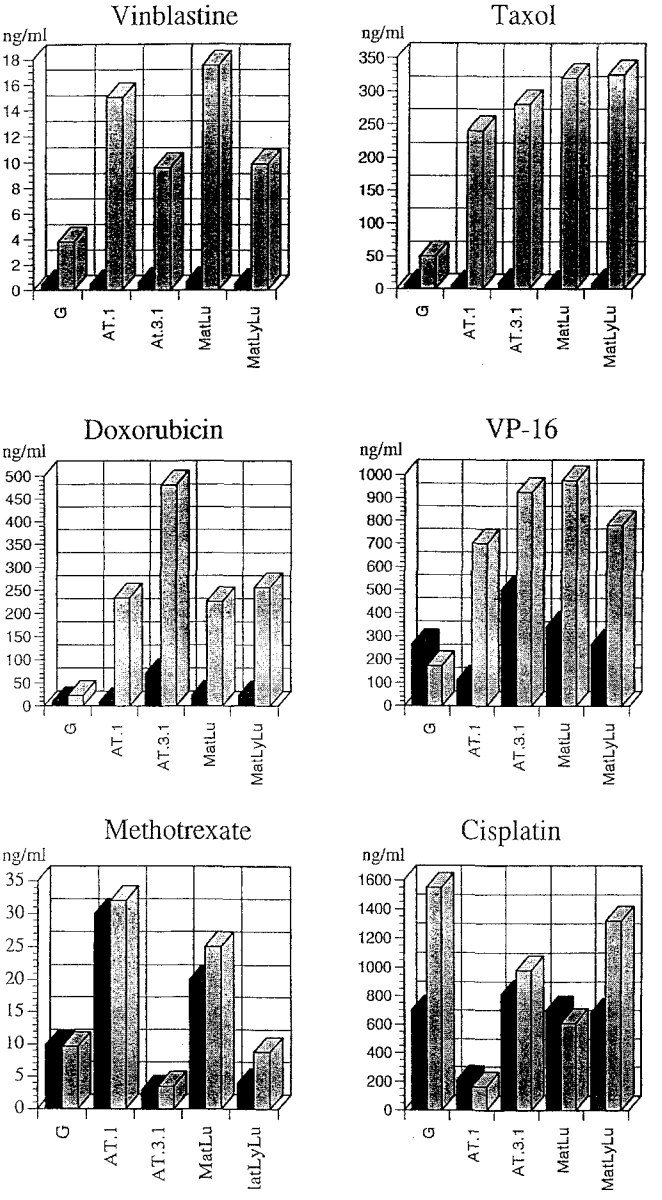

Fig. 1 IC_{50} values of the six tested cytotoxic drugs with and without the addition of verapamil

VP-16

Overall, the P-glycoprotein substrate VP 16 showed a lower efficacy than doxorubicin and taxol (IC_{50} values between 172 ng/ml for G cells and 975 ng/ml for MatLu cells). Again the androgen-independent growing cells were more resistant to this drug than the G cells (between 4.1- and 5.7-fold).

Methotrexate

Methotrexate also proved to be very effective against all the tested cell lines, with IC_{50} values ranging between 3.5 ng/ml (AT.3.1) and 32 ng/ml (AT.1). Two of the androgen-independent cell lines (AT.1, MatLu) showed a higher resistance than the G cells against this drug,

whereas the two others (AT.3.1, MatLyLu) were more sensitive.

Cisplatin

Cisplatin is not transported by P-glycoprotein and the resistance to this drug is based on other mechanisms. Overall this drug was not very effective in the examined prostate carcinoma cells, with IC_{50} values ranging between 1550 ng/ml for the G cells and 210 ng/ml for the AT.1 cells. The androgen-insensitive cells did not show a higher resistance, in fact the G cells were the most resistant cells against this drug. For some unknown reason, the AT.1 cells were about 3.7- to 9.4-fold more sensitive than the other cell lines.

Chemosensitization by verapamil

Addition of verapamil reduced the resistance to the P-glycoprotein substrates of all androgen-independent growing cell lines. In the androgen-sensitive G cells, the effect of verapamil was less pronounced (Table 2, Fig. 1). Taxol resistance of the androgen-independent cells was very effectively reversed by verapamil, with resistance-modifying factors in all androgen-independent cells of more than 30, compared to 8.4 in the G cells. Vinblastine resistance of the androgen-independent cells was also reversed very effectively, with RMFs between 16 in the AT.3.1 cells and 34.9 in the AT.1 cells, whereas in the G cells the RMF was 8.4. The combination of vinblastine and verapamil was the most effective, lowering the IC_{50} values in all cell lines between 0.43 and 0.66 ng/ml. Doxorubicin resistance of the androgen-independent cells was not as effectively reversed, with RMFs between 30.7 for the AT.1 cells and 6.9 for the AT.3.1 cells as well as VP-16 resistance with RMFs between 6.4 (AT.1) and 1.85 (AT.3.1). Verapamil had generally only a minor effect on the response to the non-MDR drugs cisplatin and methotrexate. With these two drugs, the RMFs of verapamil in the androgen-insensitive cells were, with one exception (MatLyLu/methotrexate), less than 2, whereas with the P-glycoprotein substrates they were greater than 2 with the exception of VP-16 in the AT.3.1 cells (Table 2).

Rhodamine-123 efflux

In this assay the rhodamine efflux in the prostate carcinoma cell lines was compared to the efflux in two control cell lines, one of which (KB-8–5) expressed a significant amount of P-glycoprotein, whereas the other was P-glycoprotein-negative (KB-3–1). Both cell lines could be loaded with rhodamine-123 effectively, when incubated in rhodamine-containing medium. After removal of the dye from the medium, KB-8–5 cells rapidly efflux rhodamine, whereas it remains in the KB-3–1 cells over the observation period (Fig. 2). All the androgen-independent growing prostate cell lines behave like the KB-8–5 cells, in that they are capable of extruding rhodamine-123, whereas the androgen-sensitive G cells are not able to efflux the dye over the whole observation period of 2 h (Fig. 3).

Table 2 Resistance-modifying factors (RMFs) of verapamil with different chemotherapeutic drugs. Abbreviations as in Table 1

	Vbl	Dox	Taxol	VP-16	Metho	CDDP
G	8.4	2.4	8.4	0.7	0.97	2.2
AT.1	34.9	30.7	50	6.4	1.1	0.8
AT.3.1	16	6.9	32.2	1.9	1.2	1.2
MatLu	26.5	9.8	64	2.9	1.3	0.9
MatLyLu	19.8	11.1	42.8	2.9	2.2	1.9

Expression of mdr1b mRNA

Figure 4 shows the results of an RT-PCR, using a set of primers derived from the rat *mdr1b* gene. The *mdr1b* gene is expressed in the androgen-independent cell lines

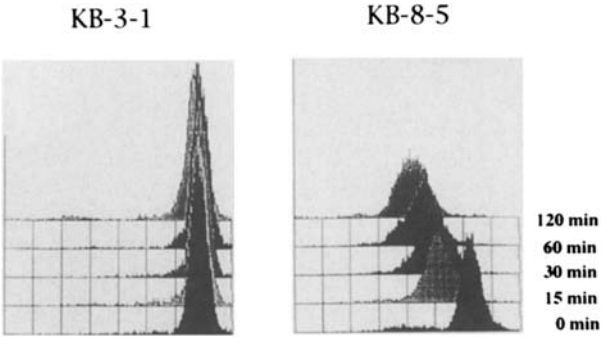

Fig. 2 Rhodamine efflux from the cell line KB-3–1 and its multidrug-resistant subline KB-8–5 over a period of 120 min. The experiment was conducted as outlined in "Material and methods"

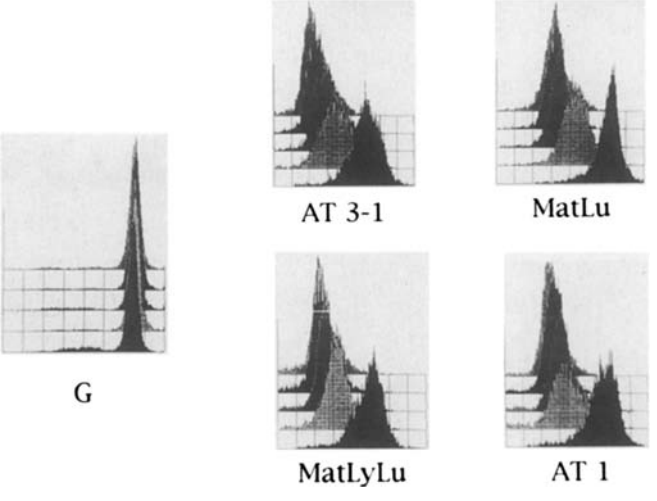

Fig. 3 Rhodamine efflux from the androgen-sensitive cell line G and the androgen-independent cell lines AT.1, AT. 3.1, MatLu, and MatLyLu

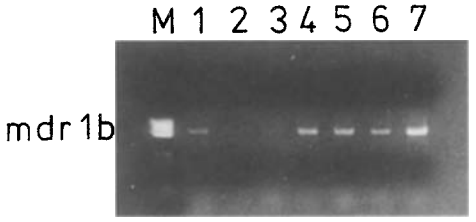

Fig. 4 RT-PCR using primers specific for the *mdr1b* gene. *Lane 1* rat liver (positive control), *lane 2* PCR with water instead of cDNA (negative control), *lane 3* G, *lane 4* AT.1, *lane 5* AT.3.1, *lane 6* MatLu, *lane 7* MatLyLu

AT.1, AT.3.1, MatLu, and MatLyLu, but not in the androgen-sensitive G cells (Fig. 4).

Discussion

We were able to demonstrate that androgen-independent growing prostate cancer cell lines, in contrast to an androgen-sensitive cell line, show an enhanced chemoresistance against a variety of certain cytotoxic drugs. These drugs are all substrates of the multidrug transporter P-glycoprotein. Two other drugs, methotrexate and cisplatin, that are known to be not transported by P-glycoprotein, were similarly effective in androgen-independent and androgen-sensitive cells. Furthermore we demonstrated that verapamil enhanced the efficacy of P-glycoprotein-substrate drugs and that only androgen-independent cells express a functionally active transporter for the fluorescent dye rhodamine-123. Finally we showed an enhanced expression of rat *mdr1b* mRNA in androgen-independent sublines compared to androgen-sensitive cells.

For our studies we used different sublines of the Dunning R3327 rat prostatic tumor. This tumor model has been used for many years in a variety of treatment studies because of its many similarities with human prostate cancer. The sublines have been established and characterized by Isaacs et al. [17] They differ widely in different characteristics. One of them (G) is androgen sensitive, whereas all the others are androgen insensitive. The expression of androgen receptors is correlated with the androgen sensitivity, in that G cells express high numbers of androgen receptors, whereas these are undetectable in all other sublines. G and AT.1 cells are nonmetastatic, whereas AT3.1, MatLu, and MatLyLu metastasize very rapidly. AT.3.1 and MatLyLu tumors metastasize lymphogenously, resulting in lymph node and lung metastases. In contrast, MatLu cells appear to metastasize via the blood, resulting exclusively in lung metastases.

Besides P-glycoprotein several other resistance mechanisms have been discovered in recent years and it is conceivable that P-glycoprotein is not the only one to protect the androgen-independent sublines from the toxic action of chemotherapeutic drugs. Multidrug-resistance-associated protein (MRP) is, like P-glycoprotein, a membrane-bound protein and belongs to the same superfamily of ATP-driven efflux pumps. Although the two proteins share only 15% of amino acid homology, they have a strikingly similar substrate specificity. MRP confers resistance to VP-16, doxorubicin, and vinblastine, but not to taxol, and the sensitivity of MRP to verapamil is lower than that of P-glycoprotein, with the result that resistance mediated by MRP can only be partially reversed with this calcium antagonist [3, 7]. The high taxol resistance we found in all androgen-independent sublines, as well as the complete reversal of vinblastine and taxol resistance by verapamil, suggest that P-glycoprotein is more important than MRP. However, we cannot rule out a role for MRP until all sublines have been examined for MRP expression.

Mutations in the topoisomerase II (*topo II*) gene or reduced activity of distinct *topo II* isoforms also result in drug resistance, especially against epipodophyllotoxins and anthracyclines. Doxorubicin and VP-16 resistance in the androgen-independent prostate carcinoma cells is likely to be mediated by a combination of different resistance mechanisms, because it cannot be reversed by verapamil as completely as vinblastine or taxol resistance. Expression analysis of the different topoisomerase isoforms should clarify their involvement in the multidrug resistance of androgen-independent tumors.

Doxorubicin exerts its cytotoxic effects not only in the nucleus, but also on the cell membrane by inducing lipoperoxides and oxygen radicals. Radical scavengers such as glutathione and associated enzymes, especially glutathione peroxidase, may be involved in doxorubicin resistance by binding and inactivating oxygen radicals. On the other hand, it has also been shown by many studies over recent years that enhanced expression of glutathione and/or its related enzymes correlates with increased resistance against cisplatin [28]. However, in our androgen-independent sublines cisplatin is more effective than in the androgen-sensitive G cells, thereby ruling out an important role of the glutathione system in the observed doxorubicin resistance.

In our experiments the multidrug resistance phenomenon was exclusively correlated with the androgen-independent state. There was virtually no difference in drug resistance between androgen-independent nonmetastatic and metastatic cell lines. As stated above, androgen-independent growing rat prostate carcinoma cells do not express androgen receptors. It might therefore be possible that the androgen receptor, a transcription factor, is involved in the regulation of *MDR* gene expression. Normally the androgen receptor binds to its ligand dihydrotestosterone in the cytoplasm and this complex is translocated to the nucleus, where it binds to androgen-responsive elements in certain gene promoters, thereby inducing, enhancing, or repressing transcription from these genes . It has been shown that castration of rats is rapidly followed by expression of different genes in the prostate, which are thought to be silenced in the presence of testosterone [19]. Some of

these are protooncogenes such as c-*myc* and c-*fos* [4]. The *Fos* protein forms together with the product of the protooncogene *jun* the transcription factor AP-1, which regulates the expression of genes that have AP-1-binding sites in their promoter regions [5]. Such an AP-1 consensus sequence has been identified in the human and mouse *MDR*1 genes, whereas it has not been described yet in the promoter region of the rat *mdr1b* gene [2, 16]. However, because the rat and mouse *mdr1b* genes have an overall sequence identity of over 90%, it is very likely that the rat *mdr1b* gene also contains an AP-1-binding site. That AP-1 has an important function in inducing transcription from *MDR* genes has been demonstrated by using ribozyme-mediated cleavage of c-*fos* mRNA to completely abrogate the drug resistance phenotype in a multidrug-resistant cell line [22]. An intact androgen-androgen receptor-complex seems necessary to inhibit AP-1 expression. In the castrated rat model the complex cannot be formed due to lack of androgen. In androgen-independent growing rat prostate cancer cell lines, the androgen receptor is not expressed. We hypothesize that AP-1 is not repressed in these cells, leading to the multidrug-resistant phenotype. Quantitative evaluation of c-*fos* and c-*jun* expression in these cell lines, as well as introduction of the androgen receptor cDNA in androgen receptor negative cells, may help to answer the question of whether the androgen receptor is involved in the regulation of the *mdr1b* gene.

Our results are supported by a recent publication about multidrug resistance in human prostate carcinoma cells [27]. It has been shown that, like in our experiments, the androgen-independent growing PC3 and DU145 cell lines are highly resistant against doxorubicin and express high amounts of P-glycoprotein, in contrast to the androgen-sensitive LNCaP cell line, which is much more drug sensitive and does not express detectable amounts of P-glycoprotein. Furthermore, like our androgen-independent cell lines, DU145 and PC3 cells do not express androgen receptors. Therefore, expression of the MDR phenotype seems to be a common phenomenon in androgen-independent growing prostate carcinoma cells from different species, and our hypothesis that the androgen receptor might be involved in the regulation of the *MDR*1 gene is supported by these experiments.

In contrast to prostate cancer cell lines, in human prostate carcinoma tissue androgen receptors have been detected by immunohistochemistry in primary and metastatic androgen-unresponsive tumors [13, 32]. Due to the lack of androgens, however, no androgen-androgen-receptor complexes can be formed that could act as transcription factors. If our hypothesis is correct, this would mean that also in human androgen-independent growing prostate carcinomas the *MDR*1 expression should be elevated.

In human prostatic carcinoma tissue, the role of *MDR*1 expression has not been established yet. Fojo et al. [9] reported low levels of *MDR*1 mRNA in normal prostate and Van der Valk et al. [33] detected P-glycoprotein expression in prostate epithelial cells by immunohistochemistry. Goldstein et al. [10] examined two carcinomas by a relatively insensitive RNA slot blot method and could not detect *MDR*1 expression in either. We have so far examined 35 prostate samples of different origin with a much more sensitive RT-PCR method, and found *MDR*1 mRNA in 31 of them, suggesting that low-level *MDR*1 expression is common in normal prostate and prostate carcinomas [24, unpublished data]. It will now be necessary to examine the expression of P-glycoprotein in a larger number of androgen-insensitive and androgen-dependent prostate carcinoma samples of different stages and grades by immunohistochemistry to further evaluate the role of this protein in prostate carcinoma drug resistance, since it has been demonstrated that low-level expression of the *MDR*1 gene correlates with clinically observed drug resistance in other tumors [14].

In conclusion, we showed that a specific pattern of multidrug resistance exists in androgen-independent growing prostate carcinoma cells, which correlates with the expression of mdr1b mRNA in the same cells, suggesting that the product of this gene is at least partly responsible for the observed drug resistance. If this finding holds also for human prostatic cancer tissue this would be clinically important since it would provide a rationale for combination therapy of cytotoxic drug and chemosensitizer in androgen-independent growing prostate carcinomas.

Acknowledgement The authors wish to thank the Wilhelm Müller Foundation, Mannheim, for generous financial support.

References

1. Akman SA, Forrest G, Chu FF, Esworthy RS, Doroshow JH (1990) Antioxidant and xenobiotic-metabolizing enzyme gene expression in doxorubicin-resistant MCF-7 breast cancer cells. Cancer Res 50:1397
2. Bhushan A, Abramson R, Chiu JF, Tritton TR (1992) Expression of c-*fos* in human and murine multidrug-resistant cells. Mol Pharmacol 42:69
3. Breuninger LM, Paul S, Gaughan K, Miki T, Chan A, Aaronson SA, Kruh GD (1995) Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution. Cancer Res 55:5342
4. Buttyan R, Zakeri Z, Lockshin R, Wogemuth D (1988) Cascade induction of c-*fos*, c-*myc* and heat shock 70k transcripts during regression of the rat ventral prostate gland. Mol Endocrinol 2:650
5. Chiu R, Boyle WJ, Meek J, Smeal T, Hunter T, Karin M (1988) The c-*fos* protein interacts with c-*jun*/AP-1 to stimulate transcription of AP-1 responsive genes. Cell 54:541
6. Chomzynzki P, Sacchi N (1987) Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 167:156
7. Cole SPC, Downes HF, Slovak ML (1989) Effects of calcium antagonists on the chemosensitivity of two multidrug-resistant human tumour cell lines which do not overexpress P-glycoprotein. Br J Cancer 59:42

8. Doroshow JH, Akman S, Esworthy S, Chu FF, Burke T (1991) Doxorubicin resistance conferred by selective enhancement of intracellular glutathione peroxidase or superoxide dismutase content. Free Radical Res Commun 12–13:779
9. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci USA 84: 265
10. Goldstein LJ, Galski H, Fojo A, Willingham MC, Lai S-L, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, Grant C, Lieber M, Cossman J, Gottesman MM. Pastan I (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81:116
11. Gottesman MM (1993) How cancer cells evade chemotherapy Sixteenth Richard and Hinda Rosenthal Foundation award lecture. Cancer Res 53:747
12. Gottesman MM, Pastan I (1993) The biochemistry of P-glycoprotein-mediated multidrug resistance. Ann Rev Biochem 62: 385
13. Hobisch A, Culig Z, Radmayr C, Bartsch G, Klocker H, Hittmair A (1995) Distant metastases from prostate carcinoma express androgen receptor protein. Cancer Res 55:3068
14. Holzmayer TA, Hilsenbeck S, Von Hoff DD, Roninson IB (1992) Clinical correlates of MDR 1 (P-glycoprotein) gene expression in ovarian and small-cell lung carcinomas. J Natl Cancer Inst 84:1486
15. Huff AC, Ward RE, Kreuzer KN (1990) Mutational alteration of the breakage/resealing subunit of bacteriophage T4 topoisomerase confers resistance to antitumour agent m-AMSA. Mol Gen Genet 221:27
16. Hsu SI, Cohen D, Kirschner LS, Lothstein L, Hartstein M, Horwitz SB (1990) Structural analysis of the mouse *mdr1a* (P-glycoprotein) promoter reveals the basis for differential transcript heterogeneity in multidrug resistant J 774.2 cells. Mol Cell Biol 10:3569
17. Isaacs JT, Isaacs WB, Feitz WFJ, Scheres J (1986) Establishment and characterization of seven Dunning rat prostatic cancer cell lines and their use in developing methods for predicting metastatic abilities of prostatic cancers. The Prostate 9:261
18. Kelley SL, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS (1988) Overexpression of metallothionein confers resistance to anticancer drugs. Science (Washington DC) 241:1813.
19. Leger JG, Montpetit ML, Tenniswood MP (1987) Characterization and cloning of androgen repressed mRNAs from rat ventral prostate. Biochem Biophys Res Commun 196
20. Mickisch GH, Merlino GT, Galski H, Gottesman MM, Pastan I (1991) Transgenic mice that express the human multidrug resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. Proc Natl Acad Sci USA 88: 547
21. Nooter K, Oostrum R, Deurloo J (1987) Effects of verapamil on the pharmacokinetics of daunomycin in the rat. Cancer Chemother Pharmacol 20:176
22. Scanlon KJ, Ishida H, Kashani-Sabet M (1994) Ribozyme-mediated reversal of the multidrug-resistant phenotype. Proc Natl Acad Sci U S A 91:11123
23. Shinoda H, Inaba M, Tsuruo T(1989) *In vivo* circumvention of vincristine resistance in mice with P388 leukemia using a novel compound, AHC-52. Cancer Res 49:1722
24. Siegsmund MJ, Cardarelli C, Aksentijevich I, Pastan I, Gottesmann MM (1994) Ketoconazol effectively reverses multidrug resistance in highly resistant KB cells. J Urol 151:485
25. Slater LM, Sweet P, Stupecky M, Wetzel MW, Gupta S (1986) Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. Br J Cancer 54:235
26. Thew KD (1994) Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 54:4313
27. Theyer G, Schirmböck M, Thalhammer T, Sherwood ER, Baumgartner G, Hamilton G (1993) Role of the MDR-1 encoded multiple drug resistance phenotype in prostate cancer cell lines. J Urol 1544
28. Timmer-Bosscha H, Timmer A, Meijer C, de Vries EG, de Jong B, Oosterhuis JW, Mulder NH (1993) *Cis*-diamminedichloroplatinum(ii) resistance in vitro and in vivo in human embryonal carcinoma cells. Cancer Res 53:5707
29. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1981) Overcoming of vincristine resistance in P388 leukemia *in* vivo and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967
30. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1982) Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res 42:4730
31. Twentyman PR (1988) Modification of cytotoxic drug resistance by non-immunosuppressive cyclosporins. Br J Cancer 58:254
32. Van der Kwast TH, Schalken J, Ruizeveld de Winter JA, van Vroonhoven CCJ, Mulder E, Boersma W, Trapman J (1991) Androgen receptors in endocrine therapy-resistant human prostate cancer. Int J Cancer 48:189
33. Van der Valk P, van Kalken CK, Ketelaars H, Broxterman HJ, Scheffer G, Kuiper CM, Tsuruo T, Lankelma J, Meijer CJLM, Pinedo HM, Scheper RJ (1990) Distribution of multi-drug resistance-associated P-glycoprotein in normal and neoplastic human tissues. Ann Oncol 1:56
34. Weißbach L, Rübben H, Jellinghaus W (1989) Die Chemotherapie des Prostatakarzinoms. In: Nagel R (ed) Aktuelle Standortbestimmung der konservativen Therapie des Prostatakarzinoms. De Gruyter, Berlin p 175
35. Yagoda A, Petrylak D (1993) Cytotoxic chemotherapy for advanced hormone -resistant prostate cancer. Cancer 71: 1098