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Multidrug Resistance in Rat Colon Carcinoma Cell Lines CC531, CC531^{mdr+} and CC531^{rev}

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A rat colon carcinoma cell line, CC531, was exposed to stepwise increasing concentrations of colchicine. A cell line, CC531^{mdr+}, which grows in the presence of 0.2 μM of colchicine was obtained. A revertant cell line, CC531^{rev} was isolated upon colchicine withdrawal. The CC531^{mdr+} displayed a multidrug-resistant phenotype. Marked resistance to the selecting agent colchicine, was found (RF = 37.5) as well as to vinblastine (RF = 11.3) and actinomycin D (RF = 2.6). Cross resistance to doxorubicin (RF = 8) and daunorubicin (RF = 13.3) was demonstrated. Verapamil was able to reverse drug resistance to colchicine and daunorubicin. The revertant cell line, CC531^{rev}, showed increased sensitivity to colchicine (RF = 0.43), vinblastine (RF = 0.13), doxorubicin (RF = 0.28) and daunorubicin (RF = 0.56). Marked cross resistance to *cis*-platinum (RF = 6.9) was also induced in CC531^{mdr+} and was maintained in CC531^{rev}. We conclude that CC531 displays an intrinsic low-level multidrug-resistant phenotype, which was amplified in the CC531^{mdr+} variant. This correlates with a higher level of expression of P-glycoprotein. CC531^{rev} lacks the multidrug-resistant phenotype and can be used as the drug-sensitive counterpart of the latter two cell lines. Furthermore, it has been shown that in these cell lines *cis*-platinum resistance is mediated through a mechanism independent of the multidrug-resistant phenotype, since the revertant cell line CC531^{rev} has lost the multidrug-resistant phenotype while retaining the concomitantly induced *cis*-platinum resistance of the multidrug-resistant variant CC531^{mdr+}.

Key words: Multidrug resistance — P-gp — Colon carcinoma — Rat cell line — CDDP-resistance

Drug resistance is a major problem in the treatment of cancer. In cancer cell lines, resistance to a variety of apparently unrelated cytotoxic agents can be induced *in vitro* by selection with a single chemotherapeutic drug (multidrug resistance, MDR). The cell lines accumulate the drugs to which they are resistant less efficiently than do their drug-sensitive counterparts. This is related to an increase in the drug efflux rate. The most consistent finding in multidrug-resistant cell lines is the overexpression of a 170 kDa membrane glycoprotein, termed P-glycoprotein (P-gp).[1–4] Drugs that are subject to the MDR phenomenon, such as vinca alkaloids, epipodophyllotoxins, anthracyclins, actinomycin D and colchicine are bound by P-gp, as are drugs which can reverse the multidrug-resistant phenotype, e.g. verapamil, diltiazem and quinidine.[5–8] The binding is energy-dependent: it requires ATP-hydrolysis. Purification of the protein by affinity chromatography made it possible to demonstrate the Mg^{2+}-dependent ATPase activity of the molecule, which might be coupled to the active efflux of anticancer drugs.[9] P-Glycoprotein is believed to act as an energy-dependent drug efflux pump, pumping out cytotoxic drugs and hence reducing their intracellular residence time.[10–16]

The pharmacological properties of P-gp, together with its distribution in normal tissues has led to the hypothesis that P-gp plays a role in detoxification mechanisms of the body by transferring potentially harmful substances from the internal compartment (intracellular and interstitial) of the body to the external compartment, i.e. the lumen of secretory organs (kidney and gastrointestinal tract). Furthermore it is believed to be an active component of the blood-tissue barrier in the brain and testicles, serving as a defense mechanism to protect these vital organs.

In vivo P-gp expression is found intrinsically in many but not all untreated tumors of organs primarily expressing P-gp (e.g. adrenal gland, colon, kidney, liver, pancreas, carcinoid tumors and chronic myelogenous leukemia in blast crisis, i.e. intrinsic MDR). In some cases P-gp is expressed in tumors that relapse after chemotherapy (acquired MDR), e.g., in acute lymphocytic leukemia, acute nonlymphocytic leukemia, breast cancer, neuroblastoma, pheochromocytoma and nodular poorly differentiated lymphoma.[2, 17–28]

In this paper we describe the selection and characterization of a multidrug-resistant rat colon carcinoma cell line, CC531^{mdr+} and its revertant CC531^{rev}. These cell lines provide us with a model for multidrug resistance which can be used both *in vivo* in syngeneic rats, and *in vitro*.

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MATERIALS AND METHODS

Cell lines The rat colon cancer cell line CC531 was obtained from the University of Leiden, the Netherlands, through P. Kuppen.[29] It grows as a monolayer in Dulbecco's modified Eagle's medium (DMEM) substituted with 5% fetal calf serum (FCS), aspartic acid (0.1 m*M*) and glutamic acid (0.3 m*M*). Cultures were maintained in the absence of antibiotics and checked at regular intervals for contamination with mycoplasmata using a nucleic acid hybridization assay (Mycoplasma T.C. rapid detection system, GEN-PROBE Inc.). The multidrug-resistant variant was continuously exposed to 0.2 μM colchicine (Sigma Chemical Company), which was added to the medium after every subculture.

MTT-assay The MTT assay was slightly modified from the method of Mosmann[30] and Carmichael *et al.*[31] Briefly, cell suspensions were obtained by trypsinization for 3–5 min with 0.25% trypsin-EDTA. The suspensions were washed three times and diluted in the incubation medium (DMEM+5% FCS+the appropriate concentration of drug). One thousand five hundred cells were seeded per well in 48-well tissue culture clusters (Costar) and incubated for 4 days at 37°C in a humidified atmosphere. Thereafter cells were incubated with 150 μl of the MTT solution (2 mg/ml) for 4 h at 37°C in a humidified atmosphere. The supernatant was gently removed by aspiration and the crystals were dissolved in 250 μl of dimethylsulfoxide (DMSO). The solutions were transferred to 96-well plates in order to read the absorbance at 570 nm in an automated microtiter reader. The 50% inhibitory doses (ID_{50}'s) were determined from the survival curves. Each point of these curves is the mean of six measurements. Every experiment was carried out in triplicate. Resistance factors (RF's) for the cytostatic drugs were calculated by dividing the mean ID_{50} of the resistant cell line by the ID_{50} of the parental cell line. The sensitization ratio (SR) was calculated by dividing the mean ID_{50} of a cell line for a particular drug by the ID_{50} of the cell line for the drug in the presence of verapamil.

Incubation and staining procedures For flow cytometric analysis, labeling with the monoclonal antibody (MoAb) C-219 (Centocor) was performed as follows: cell suspensions were obtained by trypsinization for 3–5 min with 0.25% trypsin-EDTA. Cells were fixed with 70% methanol for 5 min at −20°C. After three washings with phosphate-buffered saline (PBS), cells were resuspended in PBS with 1% bovine serum albumin (BSA) to a concentration of 10^6 cells/ml. Aliquots of 200 μl were reacted with 20 μl of C-219-FITC (50 μg/ml; Centocor) for 60 min at 4°C. Nonspecific staining, as a control, was performed with 20 μl of LgG_{2a}-FITC (50 μg/ml; Centocor). After incubation, cells were washed three times in PBS with 1% BSA and finally resuspended to a concentration of 10^5 cells/ml.

Cell suspensions for flow cytometry were processed simultaneously for peroxidase immunocytochemistry with the MoAb C-219. Cell suspensions were diluted to 200,000 cells/ml. An aliquot of 100 μl was transferred onto a polylysine-coated (1 mg/ml) microscope carrier glass. The cells were air-dried and fixed with acetone for 10 min at 0°C (on ice). After drying, cells were incubated for 10 min with 250 μl of normal horse serum (diluted 1:20 with PBS). The excess was thereafter removed and the slides were incubated with 100 μl of a C-219 solution (100 μl/ml, diluted 1:10; 1:20; 1:100 in PBS containing 1% BSA) overnight at room temperature. A nonspecific IgG_2 was used as the control antibody. The next day, slides were washed with PBS and 100 μl of biotinylated second stage reagent (diluted 1:200 with PBS containing 1% BSA) was added for 30 min. Slides were washed with PBS containing 1% BSA, and 100 μl of ABC complex (solution A diluted 1:200, solution B diluted 1:100) was added. Slides were washed and stained for 15 min with an aminoethylcarbazole (AEC) solution (20 mg of AEC, 24 ml of DMSO, 200 ml of 0.02 *M* sodium acetate, and 4 ml of 0.3% H_2O_2). Slides were rinsed with tap water and counterstained with hematoxylin for 45 s.

For flow cytometric detection of MDR by measuring the retention of DNR, cell suspensions were incubated with DNR (Sigma Chemical Company) as described by Krishan and Ganapathi,[32] Herweijer *et al.*,[33,34] Nooter *et al.*[35] and Ross *et al.*[36] Briefly, incubation was done for 1 h with 1 μg/ml DNR in full growth medium. Cells were then cooled rapidly and kept on ice. Analysis on the flow cytometer was done in the incubation medium. Cells incubated with PBS only served as controls. Verapamil (Knoll) at a final concentration of 6.6 μg/ml was added to the incubation medium in MDR-reversing experiments.

Staining for DNA content was carried out using the detergent-trypsin method described by Vindeløv *et al.*[37] Normal lymphocytes from a healthy rat were used as a diploid marker. These lymphocytes were isolated from a fresh blood sample by density gradient centrifugation (Lymphocyte Separation Medium, Flow Laboratories). They were added to cell suspensions from the rat colon cancer cell lines and all cells were stained with propidium iodide as described by Vindeløv *et al.*[37]

Flow cytometry Flow cytometric measurements were performed on a FACStar^{PLUS} (Becton Dickinson) instrument equipped with a 4 W argon-ion laser (Spectra Physics model 2025) tuned to 488 nm with 300 mW power. Green and orange fluorescence pulses were collected through 530/30 nm and 575/26 nm bandpass filters respectively. Photomultiplier pulses were amplified logarithmically. Red fluorescence, for DNA-content

measurements, was detected through a 630 nm long-pass filter and photomultiplier pulses were amplified linearly.

Statistical methods Statistical analysis was performed with the Complete Statistical System, CSS (StatSoft) on an IBM PS/2 computer. The means of the ID_{50}'s were compared using Student's t test. The criterion of statistical significance was set at $P<0.005$.

RESULTS

History The parental CC531 colon carcinoma cell line is an experimental cell line, chemically induced with 1,2-dimethylhydrazine (DMH) in the WAG rat.[30] It can be cultured *in vivo* as well as *in vitro*. It was exposed *in vitro* to stepwise increasing concentrations of colchicine.[38] The doses were chosen in such a way that a significant kill of the cell was present on routine microscopic examination. When the surviving cells had reached the same growth rate as the parental cell line, the dose of the selecting drug in the incubation medium was increased. After a period of 2 years a variant was obtained which could grow in the presence of 0.2 μM colchicine. Some of these cells were maintained in culture in the presence of colchicine and termed CC531^{mdr+}, whereas another part was cultured in the absence of the drug. Characteristics of the latter were established after six months and the cell line was subsequently termed CC531^{rev}.

Multidrug resistance phenotype Drug sensitivities for all cell lines were tested using the MTT assay. Cells were incubated with a range of different concentrations of drug(s) and allowed to grow for a period of 4 days in the presence of drug. Survival curves were plotted. The ID_{50} was calculated using linear regression analysis in the linear region of these survival curves. Resistance factors were calculated by dividing the ID_{50} of the tested cell line by the ID_{50} of the reference cell line. Sensitization ratios (SR) were calculated by dividing the mean ID_{50} of a cell line for a particular drug by the ID_{50} of this cell line for this drug in the presence of verapamil.

Compared to the parental cell line, the CC531^{mdr+} displayed a multidrug-resistant phenotype. This cell line showed marked resistance to the selecting agent colchicine (RF=37.5) as well as to vinblastine (RF=11.3) and actinomycin D (RF=2.6). Cross resistance to the anthracyclines doxorubicin (RF=8) and daunorubicin (RF=13.3) was demonstrated. The CC531^{rev} cell line was most sensitive to the agents involved in the multidrug-resistant phenotype (Table I). The parental cell line CC531 has a low level of resistance to these drugs when compared with the revertant cell line ($RF_{colchicine}=2.3$, $RF_{vinblastine}=8$, $RF_{doxorubicin}=3.6$, and $RF_{daunorubicin}=1.8$) (Table II). This resistance is amplified in the CC531^{mdr+} variant ($RF_{colchicine}=87.2$, $RF_{vinblastine}=90.2$, $RF_{doxorubicin}=29$, $RF_{daunorubicin}=23.9$, $RF_{actinomycin\ D}=2.2$) (Table II).

Table I. Mean ID_{50}'s of the CC531^{mdr+} and CC531^{rev} Cell Lines Compared to Those of the CC531 Cell Line

Drug[a]	CC531 $ID_{50}\pm SD$ (μM)[b]	CC531^{mdr+} $ID_{50}\pm SD$ (μM)[b]	CC531^{rev} $ID_{50}\pm SD$ (μM)[b]
Colchicine	0.151±0.025	5.675±0.151 (37.5)	0.065±0.210 (0.43)
Vinblastine	0.032±0.019	0.361±0.067 (11.3)	0.004±0.001 (0.13)
Daunorubicin	0.088±0.011	1.173±0.538 (13.3)	0.049±0.024 (0.56)
Doxorubicin	0.357±0.056	2.865±0.565 (8)	0.099±0.031 (0.28)
Actinomycin D	0.053±0.023	0.139±0.058 (2.6)	0.062±0.030 (1.17)
cis-Platinum	0.477±0.119	3.292±0.539 (6.9)	2.429±0.319 (5.09)

Resistance factors are given in parentheses. ID_{50}'s of CC531^{mdr+} versus CC531 and CC531^{rev} versus CC531 were all significantly different with $P<0.005$.

a) Cell lines were exposed continuously to eight different concentrations of drug in a range comprising at least three log decades.

b) After four days of incubation, survival curves were plotted. The ID_{50} is the dose at which 50% growth inhibition was noted. It was calculated using linear regression analysis in the linear region of the survival curves.

Table II. Mean ID_{50}'s of the CC531 and CC531^{mdr+} Cell Lines Compared to Those of the CC531^{rev} Cell Line

Drug[a]	CC531^{rev} $ID_{50}\pm SD$ (μM)[b]	CC531 $ID_{50}\pm SD$ (μM)[b]	CC531^{mdr+} $ID_{50}\pm SD$ (μM)[b]
Colchicine	0.065±0.21	0.151±0.025 (2.3)	5.672±0.151 (87.2)
Vinblastine	0.004±0.001	0.032±0.019 (8)	0.361±0.067 (90.2)
Daunorubicin	0.049±0.024	0.088±0.011 (1.8)	1.173±0.538 (23.9)
Doxorubicin	0.0987±0.031	0.357±0.056 (3.6)	2.865±0.565 (2.9)
Actinomycin D	0.062±0.030	0.053±0.023 (0.85)	0.139±0.058 (2.2)
cis-Platinum	2.429±0.319	0.477±0.119 (0.19)	3.292±0.539 (1.3)

Resistance factors are given in parentheses. ID_{50}'s of CC531^{mdr+} versus CC531 and CC531^{rev} versus CC531 were all significantly different with $P<0.005$.

a) Cell lines were exposed continuously to eight different concentrations of drug in a range comprising at least three log decades.

b) After four days of incubation survival curves were plotted. The ID_{50} is the dose at which 50% growth inhibition was noted. It was calculated using linear regression analysis in the linear region of the survival curves.

Table III. Mean ID_{50}'s of the CC531 Cell Lines for Colchicine, Daunorubicin and *cis*-Platinum Compared to Those of the Same Cell Lines in the Presence of Verapamil

	ID_{50}[a] Colchicine	ID_{50}[b] Colchicine/verapamil	SR[c]	*P*-value[d]
CC531	0.151±0.025	0.041±0.019	3.7	<0.005
CC531^{mdr+}	5.672±0.151	0.181±0.038	31	<0.005
CC531^{rev}	0.065±0.021	0.043±0.017	1.5	0.147
	ID_{50} Daunorubicin	ID_{50} Daunorubicin/verapamil	SR	
CC531	0.088±0.011	0.030±0.003	3	<0.005
CC531^{mdr+}	1.173±0.538	0.057±0.016	20	<0.005
CC531^{rev}	0.049±0.024	0.022±0.010	2	0.010
	ID_{50} *cis*-Platinum	ID_{50} *cis*-Platinum/verapamil	SR	
CC531	0.477±0.119	0.816±0.171	0.6	<0.005
CC531^{mdr+}	3.292±0.539	2.761±0.454	1.2	0.897
CC531^{rev}	2.429±0.319	2.462±0.415	0.98	0.965

a) Cell lines were exposed continuously to eight different concentrations of drug in a range comprising at least three log decades. After four days of incubation, survival curves were plotted. The ID_{50} is the dose at which 50% growth inhibition was noted. It was calculated using linear regression analysis in the linear region of the survival curves.
b) Cell lines were co-incubated with 6.6 μM verapamil.
c) The sensitization ratio (SR) was calculated by dividing the mean ID_{50} of a cell line for a particular drug by the ID_{50} of this cell line for this drug in the presence of verapamil.
d) Student's *t* test.

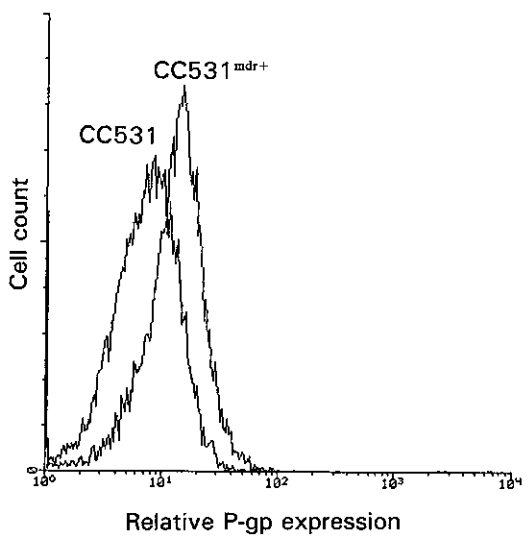

Fig. 1. P-Glycoprotein expression of the CC531 and CC531^{mdr+} cell lines measured flow cytometrically using the MoAb C-219. Each histogram represents the fluorescence distribution for 10,000 cells of the respective cell lines. Controls were performed with a nonspecific IgG_2.

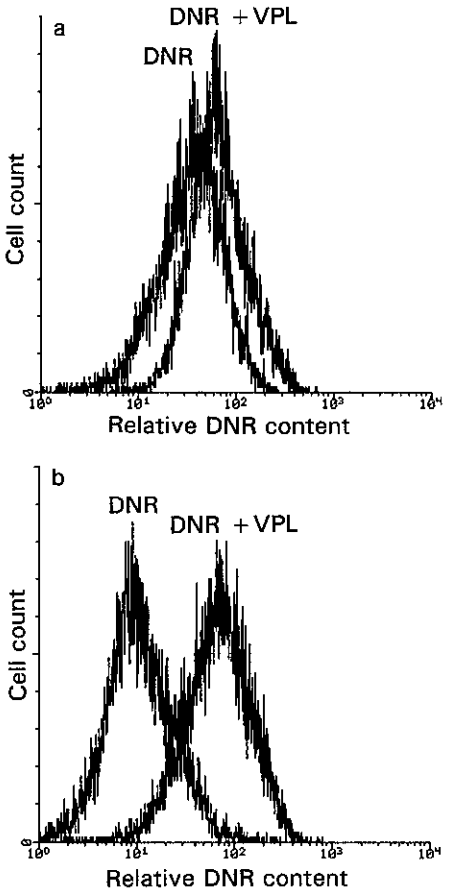

Fig. 2. DNR accumulation in the absence (DNR) and presence (DNR+VPL) of 6.6 μM verapamil for the CC531 (a) and the CC531^{mdr+} (b) cell lines. Each histogram represents the fluorescence distribution for 10,000 cells of the respective cell lines.

Verapamil in a concentration of 6.6 μM, was able partially to reverse the resistance to colchicine and daunorubicin (SR=31 and 20, respectively). No significant effect of verapamil was noted in the CC531^{rev} variant (Table III).

Apparently resistance to *cis*-platinum (RF=6.9) was induced in the multidrug-resistant CC531^{mdr+} cell line during its selection (Table I). In contrast to the multidrug-resistance phenotype, resistance to *cis*-platinum was maintained in the revertant cell line CC531^{rev} (Table I). Incubation in the presence of verapamil had no significant effect on *cis*-platinum resistance in these cell lines (Table III).

Growth characteristics Using the MTT-assay, growth curves for the CC531, CC531^{mdr+}, CC531^{rev} cell lines were assessed. All cell lines had similar growth character-

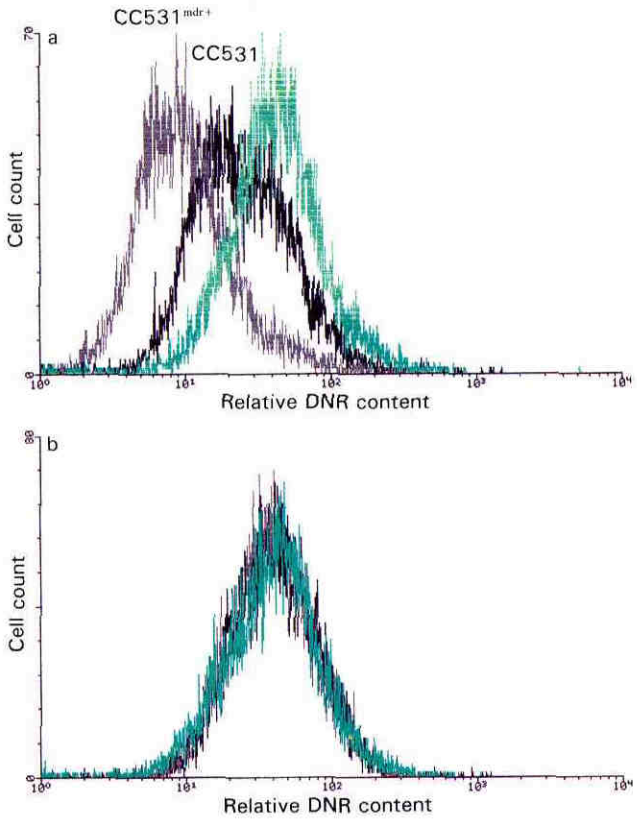

Fig. 3. DNR accumulation in the absence (a) and presence (b) of 6.6 μM varapamil for the CC531, the CC531^{mdr+} and the CC531^{rev} cell lines. Each histogram represents the fluorescence distribution for 10,000 cells of the respective cell lines.

istics with comparable doubling times ranging between 34 and 38 h.

DNA content The DNA content of the cell lines was determined by using the detergent-trypsin method of Vindeløv.[37] As a diploid marker, normal rat lymphocytes were used. DNA indices were obtained by calculating the ratio of mean fluorescence channels of G_0/G_1 phase of the tumor cells over G_0/G_1 of the normal lymphocytes. The parental cell line CC531 was found to be tetraploid (DNA index = 1.99). A significant amount of DNA was lost from this cell line during the selection of the CC531^{mdr+} variant, since the latter was found to be hypotetraploid (DNA index = 1.77). The revertant cell line CC531^{rev} was found to be tetraploid (DNA index = 2.00) (data not shown).

Reactivity to the MoAb C-219 In both immunohistochemical examination and flow cytometric assay, reactivity of the monoclonal antibody C-219 with these cell lines was established. A weak reactivity of the parental cell line CC531 with C-219 was found. This reactivity was increased in the CC531^{mdr+} variant (Fig. 1). No reactivity with C-219 was found in the CC531^{rev} cell line.

Measurement of DNR content A flow cytometric assay was used to measure the accumulation of anthracyclines in the cells. Daunorubicin was used as the fluorescent marker of choice. Compared to the parental cell line, CC531, the CC531^{mdr+} variant displays a weaker fluorescence. Addition of verapamil at an inhibitory dose (6.6 μM) during the incubation period increased fluorescence in both the parental line, CC531, and the CC531^{mdr+} variant (Fig. 2a and 2b). The increase in fluorescence was the most intense in the CC531^{mdr+} cells. The revertant cell line CC531^{rev} displayed the highest levels of fluorescence with daunorubicin (Fig. 3a). Verapamil, in the concentration used, had no effect on DNR accumulation in this cell line. Addition of verapamil at an inhibitory dose (6.6 μM) during the incubation period increased fluorescence in CC531^{mdr+}, CC531^{rev} and CC531 to such an extent that all cell lines attained the same level of fluorescence (Fig. 3b).

DISCUSSION

The present report describes the selection and phenotypical characterization of a multidrug-resistant cell line, CC531^{mdr+}, and the drug-sensitive revertant cell line CC531^{rev} from a rat colon carcinoma cell line, i.e., CC531. Multidrug resistance was induced in this cell line by continuous incubation with stepwise increasing concentrations of colchicine, a well known inducer of the phenotype.[39-41] The CC531^{mdr+} cells display the classical multidrug-resistant phenotype with resistance to the selecting agent colchicine and cross resistance to daunorubicin, doxorubicin, vinblastine and actinomycin D (Table I). The resistance can be reversed partially by verapamil, a calcium antagonist and inhibitor of the multidrug-resistant phenotype as first described by Tsuruo *et al.*[42, 43] (Table III). By culturing in the absence of the selecting drug, a revertant cell line was isolated. This CC531^{rev} cell line has regained sensitivity to the multidrug resistance-related drugs (Table I). Moreover the revertant cell line displays higher sensitivity to the agents of interest when compared to the parental cell line CC531. Differences between drug sensitivity of the cell lines included in the present study are not due to differences in growth rate since all cell lines appeared to have approximately the same doubling times (data not shown). Using the detergent-trypsin method of Vindeløv, the DNA-ploidy of the cell lines was determined. Both the parental and the revertant cell lines are tetraploid, but the CC531^{mdr+} cell line is hypotetraploid. A considerable amount of DNA has thus been lost from the latter cell line in the process of selection at increasing levels of

colchicine. Differences in DNA-ploidy between the cell lines warrant further investigation.

Using both immunohistochemistry and flow cytometry, we examined the expression of the multidrug transporter P-gp in our cell lines. We found that the parental cell line CC531 displayed a weak immunoreactivity to the monoclonal antibody C-219. This is concordant with reports of intrinsic expression of P-gp on normal colon and colon carcinoma cells.[18, 44–46] The multidrug-resistant variant displays an enhanced immunoreactivity to C-219 compared to the parental cell line. No immunoreactivity to C-219 was detected in the revertant cell line CC531^{rev}. These data correspond very well to the drug-sensitivity data.

The level of drug resistance correlates inversely with the level of fluorescence with daunorubicin. The most sensitive cell line, CC531^{rev}, displayed the highest fluorescence with the anthracycline, while the level of fluorescence was lower in the parental CC531 cell line. The most drug-resistant variant, CC531^{mdr+}, has the lowest level of fluorescence. Recently, a report has been published, describing a drug-resistant rat mammary carcinoma cell line, MatB 13762.[47] The cell line can be grown *in vivo* as a solid tumor in Fischer 344 rats, as well as *in vitro*. The Adr^{R} cell line has been rendered highly drug-resistant by *in vitro* selection with adriamycin. It displays a 200-fold resistance to adriamycin, a 230-fold resistance to vincristine, a 2-fold resistance to melphalan and a 2.4-fold resistance to BCNU. Since mammary tissue does not show intrinsic expression of P-gp, the cell line is a model for acquired drug resistance. The isolation of the three cell lines described here has provided an interesting *in vitro* model for multidrug resistance in a rat colon carcinoma cell line. Here we have a cell line, CC531, which displays the classical multidrug-resistant phenotype intrinsically, since it is derived from a carcinoma of the colon, a tissue type known to express P-gp intrinsically. By selecting in the presence of the drug colchicine the CC531^{mdr+} cell line has developed higher levels of drug resistance and can be regarded as a model for acquired multidrug resistance. The revertant cell line, CC531^{rev}, in contrast, has lost the expression of P-gp and the multidrug-resistance phenotype and can be considered as the drug-sensitive counterpart of the other cell lines (Table II). Therefore, the three cell lines offer the opportunity to study the underlying mechanisms of different level of drug resistance in the same animal cell line. Moreover, the intrinsic multidrug-resistant cell line CC531 can be cultured *in vivo* in the WAG rat as well. Preliminary experiments using the cell lines described here in WAG rats have yielded promising results and the *in vitro* model therefore may be easily transferred into an *in vivo* model.

Remarkably, resistance to *cis*-platinum (RF=6.9) was simultaneously induced in the multidrug-resistant CC531^{mdr+} cell line. To our knowledge, this is the first paper that describes the generation of multidrug resistance concomitantly with resistance to *cis*-platinum. No data are yet available about the underlying mechanism(s) in this cell line. *cis*-Platinum accumulation and DNA-adduct formation in the cells of interest are currently being investigated according to established methods.[48, 49] However, most likely resistance to *cis*-platinum is mediated through (a) mechanism(s) independent of the multidrug resistance phenotype, since in the CC531^{rev} cell line multidrug resistance is lost while *cis*-platinum resistance is conserved and so the latter is not likely to be associated with the part of DNA lost in the CC531^{mdr+} cell line. Karyotype analysis has therefore been initiated.

In summary, a new model for multidrug resistance has been developed comprising three cell lines of the same origin with different levels of multidrug resistance. The cell lines can be used in *in vitro* studies and appear promising for *in vivo* studies in syngeneic WAG rats.

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