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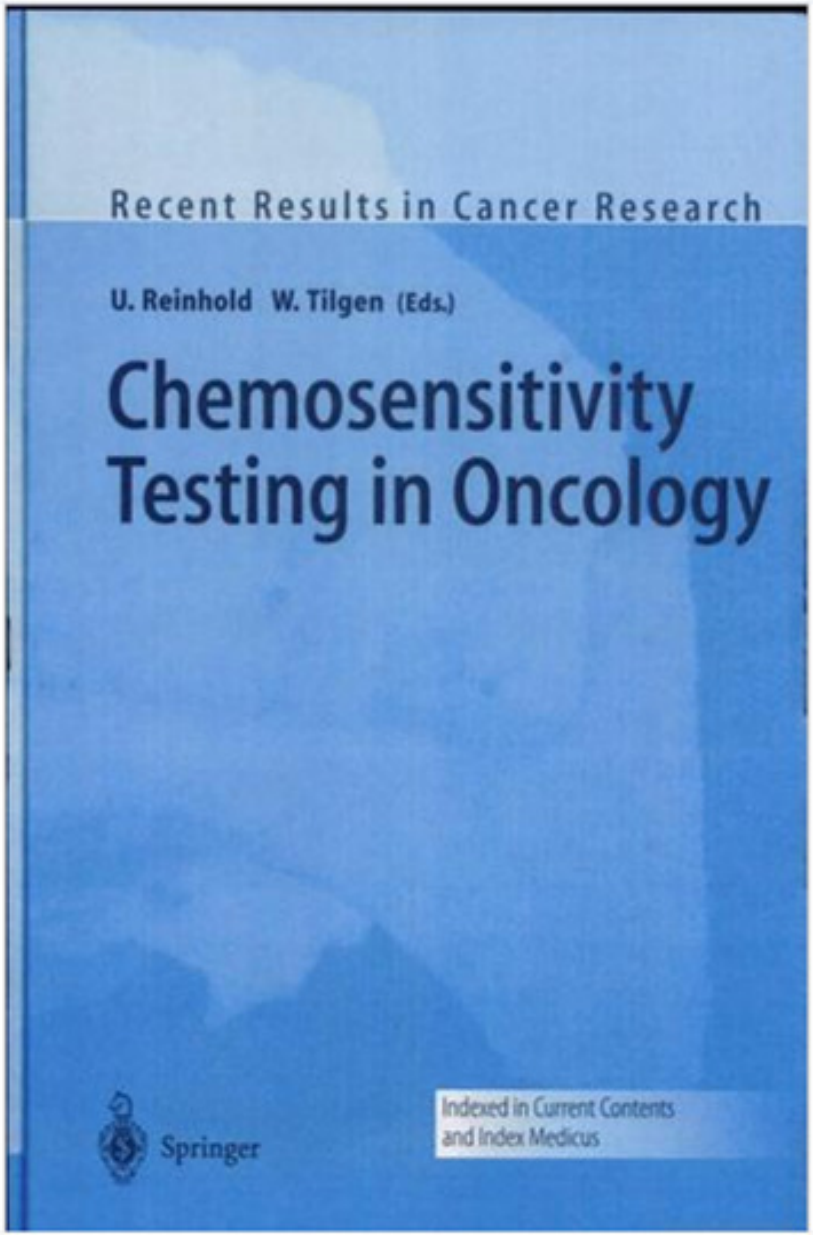

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Cisplatin, Doxorubicin and Paclitaxel Induce *mdr1* Gene Transcription in Ovarian Cancer Cell Lines

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Abstract

The clinical observation of the multidrug resistance (MDR) phenotype is often associated with overexpression of the *mdr1* gene, in particular with respect to ovarian cancer. However, until now the *mdr1*-inducing potential of commonly used antineoplastics has been only incompletely explored. We performed short-term cultures of six ovarian cancer cell lines (MZOV4, EFO27, SKOV3, OAW42, OTN14, MZOV20) exposed to either blank medium or cisplatin, doxorubicin or paclitaxel at concentrations related to the clinically achievable plasma peak concentration. A highly specific quantitative real-time RT-PCR was used to detect the *Mdr1* transcripts. *Mdr1* mRNA contents were calibrated in relation to coamplified GAPDH mRNA. *Mdr1* mRNA was detectable in each cell line. In 13 out of 18 assays (72%) the specific anticancer drug being tested induced *mdr1* transcription. No decrease in *mdr1* mRNA concentration was observed. Our data suggest that *mdr1* induction by antineoplastics is one of the reasons for failure of ovarian cancer therapy but may vary individually.

Introduction

Ovarian cancer is one of the main causes of death related to gynecological malignancy: Nearly 65% of ovarian cancer patients will die from their disease within 5 years [8]. Although ovarian carcinomas are considered highly responsive to cytotoxic treatment, they rapidly develop chemoresistance [6]. Thus the multidrug resistance (MDR) phenotype of ovarian tumor cells is one of the major obstacles to the therapy of ovarian cancer [12].

On the molecular level, increased expression of the *mdr1* gene is the best-studied mechanism for the MDR phenotype [6]. The *mdr1* gene encodes the p170 glycoprotein, a transmembrane protein that eliminates toxic agents from

the intracellular compartment and thus confers resistance to a wide variety of natural products. However, insufficient information is available concerning the regulation of the *mdr1* gene during the clinical course of a cancer patient undergoing antineoplastic chemotherapy. In ovarian cancer as well as in other neoplasms, p170 overexpression leads to the MDR phenotype and indicates a worse prognosis [8, 18].

Consequently, intensified research efforts are needed to obtain more basic data with respect to *mdr1* gene regulation in ovarian cancer [17]. The design of these studies should take into account techniques with increased sensitivity. Recently, *mdr1* gene amplification was excluded as a cause for *mdr1* overexpression in ovarian cancer [19]. Thus, in this tumor entity, p170 overexpression is more likely a result of increased transcription/translation of the *mdr1* gene. The study presented here was designed to explore whether antineoplastics are capable of inducing the *mdr1* gene. We therefore employed a *mdr1* mRNA detection assay using real-time PCR, which is more sensitive than in any other study performed so far. With this assay system the *mdr1*-inducing potency of commonly applied anticancer drugs was investigated.

Materials and Methods

Tumor Cell Culture

The ovarian cancer cell lines were kind gifts from L.G. Poels (Nijmegen, The Netherlands) (OTN14) and V. Möbus (Ulm, Germany) (MZOV4, MZOV20) or were obtained from DSMZ (Braunschweig, Germany) and DKFZ (Heidelberg, Germany). Tumor cells were grown in AIM V medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IE/ml penicillin and 100 µg/ml streptomycin at 100 cells/µl (37°C, humidified 95% air-5% CO_2 atmosphere). Cells were exposed for 3 days to either blank medium (control) or the different antineoplastic agents: doxorubicin (DOX) 0.5 µg/ml, *cis*-diamino-dichloroplatinum(II) (CDDP) 3.8 µg/ml, and paclitaxel (PCT) 13.6 µg/ml. The cytostatics assayed referred to either the clinical peak plasma concentration (PPC) after administration of an intravenous standard dose (DOX, CDDP) or the equivalent of the area under the plasma elimination curve (PCT). Each assay was performed in triplicate.

Quantitative Real-Time RT-PCR

Cells were harvested by centrifugation (5 min, 8,000 *g*), washed in phosphate-buffered saline (PBS), and resuspended in lysis buffer. Total RNA was extracted with the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and quantified with the RiboGreen RNA quantification kit (MoBiTec, Göttingen, Germany).

A *mdr1*-specific 411-bp sequence was amplified in the presence of an intrinsic fluorescein-labeled *mdr1* probe. The TaqMan EZ RT-PCR Kit (GAPDH mRNA, Applied Biosystems, Weiterstadt, Germany) was used as an internal control. The mdr/GAPDH biplex-qu-RT-PCR contained 5 ng of RNA, 300 µM dATP, dCTP, and dGTP, 600 µM dUTP, 60 nM reference dye, each primer at 200 nM, each probe at 100 nM, 0.01 U/µl AmpErase UNG, and 0.1 U/µl r*Tth* DNA polymerase in TaqMan EZ buffer (50 mM bicine, 115 mM K-acetate, 0.01 mM EDTA, 40% glycerol, 3 mM Mn-acetate, pH 8.2) in a final volume of 50 µl. Quantitative real-time RT-PCR was performed by reverse transcription for 30 min at 30°C, denaturation for 10 min at 95°C and 40 cycles of 15 s at 95°C, and annealing and elongation for 1 min at 60° C. Resulting fluorescence was detected at each PCR cycle by the ABI 7700 Sequence Detection System (Applied Biosystems) automatically. Each *mdr1* or GAPDH signal, respectively, was quantified by the specific threshold cycle number (C_T).

Results

All ovarian cancer cell lines investigated were successfully analyzed. For GAPDH and *mdr1* expression, both single quRT-PCR and biplex-quRT-PCR revealed similar absolute results, indicating reproducibility and reliability of the assay. The expression of the *mdr1* gene was indicated as a quotient of *mdr1* C_T/GAPDH C_T. The quantitative real-time RT-PCR was performed in triplicate to validate the test. For each RT-PCR the assay system produced comparable results with acceptable variation: The quotients ranged from 0.60 to 0.88 and the intra-assay deviation did not exceed 0.08.

Summarizing all control experiments of the six cell lines, the *mdr1* C_T/GAPDH C_T quotient was 0.66±0.04. Therefore, quotients of 0.62–0.70 represent a "normal" distribution and are equivalent to *mdr1* expression rates ranging between 94% and 106% in relation to the control. For further analyses, each blank medium control was set at 100%.

As summarized in Table 1, all cell lines displayed increased *mdr1* gene expression in response to some of the cytostatics. In none of the assays was downregulation of *mdr1* gene expression detected. In four out of six (66%) ovarian cancer cell lines, treatment with CDDP or DOX increased *mdr1* expression to an average of 118% of the control, ranging from 109% to 133% (CDDP) or 107% to 130% (DOX), respectively. PCT-induced *mdr1* expression was detected in five out of six (83%) ovarian cancer cell lines. The degree of PCT-induced *mdr1* mRNA augmentation ranged between 109% and 119% with a mean of 114%.

Discussion

The unsatisfactory clinical results in refractory ovarian cancer are likely traced back to an increase in expression of the *mdr1* gene [8, 11]. Further-

Table 1. Relative increase of mdr1 gene expression induced by cytostatics in ovarian cancer cell lines. Tumor cells were grown overnight with either blank medium (control) or commonly applied anticancer therapeutics. For better comparison, each control was set as 100%. Increased mdr1 expression values exceeding the "normal" distribution are indicated in bold numbers. For each value, the standard deviation is indicated

	OTN14	EFO27	MZOV4	MZOV20	SKOV3	OAW42
Cisplatin	**121**±4.3	105±1.6	**109**±0.5	**109**±2.9	101±5.7	**133**±4.6
Doxorubicin	**130**±7.2	106±4.6	**107**±0.5	101±0.5	**108**±0.8	**128**±6.2
Paclitaxel	**113**±4.0	**113**±2.8	**119**±2.3	**109**±1.3	99±1.2	**119**±3.4

more, an increase of *mdr1* mRNA is a highly predictive determinant of patients' survival [16]. Accordingly, the impact of *mdr1* expression is a major concern in basic research of the molecular biology of ovarian cancer. We thus designed a study concerning the *mdr1* mRNA increase induced by a particular anticancer drug itself, thereby investigating the role of the *mdr1* gene in the development of drug resistance.

It is reported that anticancer drugs are able to induce *mdr1* transcription [4]. Resistance against doxorubicin or paclitaxel is mediated by the *mdr1* gene in OAW42 cells [13]. Accordingly, chemoresistant ovarian cancers display high *mdr1* mRNA levels [14]. Exposure to DOX results in a fast and dramatic increase of *mdr1* gene expression in human sarcoma in vivo [1]. Consequently, and in contrast to earlier data [5, 9, 10, 22], the predictive value of *mdr1* expression has recently been shown [2]. A study performed with both primary and recurrent native ovarian carcinomas revealed that the increased *mdr1* mRNA levels may not be maintained for a longer interval [20].

In 1999, Robert hypothesized that the *mdr1* gene might be expressed at very low levels in all tumors, including ovarian cancer [17]. New basic research involving new strategies with increased sensitivity is required to investigate the role of the *mdr1* gene in development of the MDR phenotype of ovarian cancer precisely and thus to unravel the aforementioned controversial studies. Here, an assay is presented to determine the *mdr1* expression rate with a high specificity and sensitivity, which is easily incorporated into clinical routine. This study shows a detectable *mdr1* mRNA presence in all cancer cell lines, confirming the hypothesis of low but persistent *mdr1* mRNA levels in ovarian cancer [17]. Furthermore, a drug-induced augmentation of the *mdr1* transcription rate was observed in 72% of the experiments and no decrease occurred. This also holds true for CDDP exposure, although this drug is known not to be a *mdr* target but capable of selecting multidrug-resistant ovarian cancer cells exhibiting high *mdr1* levels [21]. Our data and those of the earlier studies suggest that a particular drug induces *mdr1* transcription and, consequently, supports its own extrusion out of the tumor cell. However, the response rates indicate that the extent of this phenomenon is variable and should be investigated for each single tumor separately.

Nevertheless, the assay presented here performed with native cancer cells may be useful in identifying patients who will definitely benefit from a regimen of a common chemotherapy combined with a *mdr1*-inhibiting drug. Clinical trials have been presented recently with encouraging results [3, 7, 15], but they lack a molecular definition of the tumors.

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3 Clinical Relevance of Tumor-Directed Therapy