Biomedicine & Pharmacotherapy 68 (2014) 111–117

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Original article

Expression of MDR1 and MDR3 gene products in paclitaxel-, doxorubicin- and vincristine-resistant cell lines

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ARTICLE INFO

Article history:
Received 23 August 2013
Accepted 24 September 2013

Keywords:
Drug-resistant cancer
Drug transporters
Expression levels
MDR3

ABSTRACT

Multiple drug resistance is one of the main reasons for low chemotherapeutic efficiency in cancer patients. The proteins that are most frequently implicated to play a role in this mechanism are transmembrane proteins that are members of the ABC family. The most important ABC protein is MDR1 (ABCB1), which is expressed in over fifty percent of drug-resistant cancers. The phosphatidylcholine transporter, MDR3 (ABCB4), exhibits high homology with MDR1. An increasing body of evidence suggests that MDR3 plays a role in drug resistance. In the present study, we used doxorubicin-, paclitaxel- and vincristine-resistant cancer cell lines. A chemosensitivity assay MTT test was performed to assess drug resistance. Quantitative real-time polymerase chain reaction analyses were performed to determine the mRNA expression levels of the MDR1 and MDR3 genes. We observed dose-dependent responses to doxorubicin, paclitaxel and vincristine in the investigated cell lines. In all of the drug-resistant cell lines that we studied, we observed increased MDR1 and MDR3 transcript levels. In a doxorubicin-resistant variant of the LoVo cell line (LoVoDx), MDR3 was expressed at higher levels than MDR1. We also observed high correlations between MDR3 expression and resistance to doxorubicin and paclitaxel. Our results suggest that MDR3 plays an active and important role in drug resistance in the investigated cell lines.

1. Introduction

Multidrug resistance (MDR) is a major problem in the chemotherapeutic treatment of cancer patients [1]. Some cancers exhibit significant primary resistance to cytostatic agents, while other cancers acquire the MDR phenotype after prolonged exposure to cytostatic drugs. Drug resistance can be mediated by different mechanisms. However, drug transporters from the ABC family play a central role in this process [2]. The most important ABC protein is P-glycoprotein, which is encoded by the MDR1 (ABCB1) gene [3]. Overexpression of P-gp has been directly implicated in resistance to a broad spectrum of chemotherapeutic agents, including the taxanes, the anthracyclines, and the vinca alkaloids [4–6]. For various types of cancer, accumulated evidence from numerous studies indicates that MDR1 overexpression may be the predominant factor that limits the efficacy of chemotherapeutic agents. The human MDR1 gene exhibits high nucleotide sequence homology (approximately 80%) with the MDR3 (ABCB4) gene [7,8]. MDR3 is known as a phosphatidylcholine transporter [9,10]. However, increasing evidence suggests that MDR3 plays a role in drug resistance. High levels of MDR3 gene expression have been reported in leukaemic cells from patients with prolymphocytic leukaemia (PLL) that did not express the MDR1 gene [11]. Cyclosporin A (CyA), a P-gp inhibitor, increased daunorubicin accumulation in these cells, suggesting that MDR3 plays a role in drug resistance [11]. Herweijer et al. reported that the MDR3 gene is coexpressed with the MDR1 gene in B-cell chronic lymphocytic leukaemia (B-CLL). However, in B-cell prolymphocytic leukaemia (B-PLL), only MDR3 expression has been detected [12]. Higher MDR3 expression levels in the advanced stages of chronic lymphocytic leukaemia (CLL) than in the early stages of CLL have been reported [13]. Arai et al. suggested that MDR3 expression has been negatively correlated with clinical outcome [14]. Furthermore, treatment of MDR3 positive, but MDR1 negative, cells with CyA lead to significant increases in rhodamine 123 (Rh123) at the intracellular level [14]. Expression of MDR3 together with MDR1, or independently of MDR1, has also been reported in patients with soft tissue sarcomas (STS) [15]. Furthermore, MDR3 expression was increased to a greater degree than MDR1 after chemotherapy. The expression of MDR3 has also been reported in ovarian cancer cell lines [16]. In addition, MDR3 has been shown to be able to transport MDR1 substrates in both humans [17] and mice [18].

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http://dx.doi.org/10.1016/j.biopha.2013.09.004

Smith et al. reported the increased directional transport of several MDR1-targeted chemotherapeutic agents, such as digoxin, paclitaxel, and vinblastine, through the polarised layers of MDR3-transfected cells [17]. Devault and Gros reported overlapping substrate specificities between MDR1 and MDR3 in a mouse model [18]. These results indicate that MDR3 may indeed play a role in drug resistance. However, MDR3 expression has mainly been investigated in different types of leukaemia. The role of MDR3 in cancer drug resistance remains poorly understood.

In the present study, we used a cancer model of drug resistance to determine the role of MDR3 expression in drug resistance during cancer. The objective of our research was to compare the expression of MDR1 and MDR3 in doxorubicin-, paclitaxel- and vincristine-resistant cell lines and determine the correlation between the expression of these genes and levels of resistance to doxorubicin and paclitaxel.

2. Materials and methods

2.1. Reagents

Doxorubicin, paclitaxel, vincristine, RPMI 1640 medium, EMEM medium, foetal bovine serum, an antibiotic-antimycotic solution and L-glutamine were purchased from Sigma (Saint-Louis, MO). A Cell Proliferation Kit I (MTT) was purchased from Roche Diagnostics GmbH (Mannheim, Germany).

2.2. Cell lines and cell culture

The established human colon adenocarcinoma cell line LoVo, the doxorubicin-resistant subline LoVoDX, and the human ovarian carcinoma cell line A2780 were purchased from ATCC. The primary human ovarian cancer cell line W1 was established in our department (December 2009) from tissue that had been obtained from an untreated patient who had been diagnosed with ovarian cancer.

Sublines that were resistant to doxorubicin (A2780DR1, A2780DR2, and W1DR), paclitaxel (A2780PR1, A2780PR2, W1PR1 and W1PR2) and vincristine (W1VR) were generated by exposure of the A2780 or W1 cell line to incremental increases in the concentrations of the relevant drugs. The final concentrations of each drug were 100 ng/mL of DOX, 1100 ng/mL of PAC, and 10 ng/mL of VIN. These concentrations were based on the work of Dietel et al., 1997 and were twofold greater than the concentrations in the plasma 2 hours after intravenous administration [19]. The maintenance dose of doxorubicin in the LoVoDx cell line was 200 ng/mL. All of the cell lines were maintained as a monolayer in complete medium [RPMI 1640 medium (for W1 and its drug-resistant sublines) or EMEM (for LoVo, LoVoDx, A2780 and their drug-resistant sublines) supplemented with 10% (v/v) foetal bovine serum, 2 pM L-glutamine, penicillin (100 units/mL), streptomycin (100 units/mL) and amphotericin B (25 μg/mL)] at 37 °C in a 5% CO_2 atmosphere.

2.3. Drug sensitivity assays

The drug sensitivities of the drug sensitive and resistant cell lines were confirmed using the MTT cell survival assay. Briefly, all of the cell lines were seeded at densities of 5000 cells/well in 96-well plates. The cells were allowed to grow for 72 hours and were subsequently treated with either fresh medium that had been supplemented with increasing concentrations of the drugs or fresh medium that had not been supplemented, after which point they were incubated for 72 h at 37 °C. After 72 h of exposure, 10 μL of the MTT labelling reagent was added to the medium (the final concentration of MTT was 0.5 mg/mL), and the cells were incubated for an additional 4 h period. Following this process, 100 μL of solubilisation solution was added to each well. The absorbance of each sample was measured in a microplate reader at 570 nm, with a reference wavelength of 720 nm, according to the manufacturer's recommended protocol. The negative control was conducted using cell-free culture medium containing both the MTT reagent and the solubilisation solution. The experiments were repeated three times. Cell viability is expressed as the percentage of the untreated controls (means ± SEM).

2.4. Examination of MDR1 and MDR3 gene expression using Q-PCR

Changes in MDR1 and MDR3 gene expression in the drug sensitive and drug-resistant cell lines were examined. RNA was isolated using the Gene Matrix Universal RNA Purification Kit (EURx Ltd.), as described in the manufacturer's recommended protocol. Reverse transcription was performed using M-MLV reverse transcriptase (Invitrogen), as described in the manufacturer's recommended protocol, using a thermal cycler (Veriti 96 well Thermal Cycler). Two micrograms of RNA was used to synthesise cDNA. Real-time PCR was performed using the Eppendorf PCR System (Mastercycler realplex^4), Maxima® SYBR® Green/ROX Q-PCR Master Mix (Fermentas) and sequence-specific primers, as indicated in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GADPH), β-actin, hypoxanthine-guanine phosphoribosyltransferase 1 (HRPT1) and beta-2-microglobulin (β2 M) served as the normalising genes (geometric mean) against which changes in the examined gene expression were compared. Gene

Table 1
Oligonucleotide sequences that were used for Q-PCR analyses.

Transcript	Sequence (5′–3′ direction)	ENST number http://www.ensembl.org	Product size (bp)
MDR1	TGACAGCTACAGCACGGAAG TCTTCACCTCCAGGCTCAGT	00000265724	131
MDR3	AACCCCAAGATCCTTCTGCT GGACCGTAGACAGTCGGTGT	00000265723	136
GADPH	GAAGGTGAAGGTCGGAGTCA GACAAGCTTCCCGTTCTCAG	00000229239	199
β-actin	TCTGGCACCACACCTTCTAC GATAGCACAGCCTGGATAGC	00000331789	169
HRPT1	CTGAGGATTTGGAAAGGGTG AATCCAGCAGGTCAGCAAAG	00000298556	156
β2 M	CGCTACTCTCTCTTTCTGGC ATGTCGGATGGATGAAACCC	00000558401	133