Cancer Chemother Pharmacol (2013) 71:153–163
DOI 10.1007/s00280-012-1992-x

ORIGINAL ARTICLE

Changing the expression vector of multidrug resistance genes is related to neoadjuvant chemotherapy response

Nicolay V. Litviakov · Nadezhda V. Cherdyntseva · Matvey M. Tsyganov · Evgeny V. Denisov · Evgeny Y. Garbukov · Marina K. Merzliakova · Victor V. Volkomorov · Sergey V. Vtorushin · Marina V. Zavyalova · Elena M. Slonimskaya · Vladimir M. Perelmuter

Received: 17 July 2012 / Accepted: 19 September 2012 / Published online: 2 October 2012
© Springer-Verlag Berlin Heidelberg 2012

Abstract
Purpose We aimed to examine the association between alterations in multidrug resistance (MDR) gene expression, measured before and after neoadjuvant chemotherapy (NAC), and short-term response in a cohort of stage IIA–IIIC breast cancer patients ($n = 84$).
Methods All patients were treated with two to four preoperative cycles of FAC (5-fluorouracil–adriamycin–cyclophosphamide), CAX (cyclophosphamide–adriamycin–xeloda) or taxane regimes. The expression levels of key MDR genes (*ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC5*, *ABCG1*, *ABCG2*, *GSTP1*, and *MVP*) were evaluated in both tumor tissues obtained pre-therapy and in specimens removed by final surgery, using TaqMan-based quantitative reverse transcriptase PCR.
Results No significant difference in the average level of MDR gene expression in paired breast tumors before and after NAC was found when analyzed in both responsive and non-responsive patients. There was no correlation between the expression levels of MDR genes in pre-NAC tumors and immediate NAC response. In the group with tumor responses, we found a statistically significant downregulation of expression of *ABCB1*, *ABCC1, ABCC2*, *ABCC5, ABCG1*, *ABCG2*, *GSTP1*, and *MVP* genes following NAC in FAC and CAX-treated patients (67–93 % of cases). In contrast, we found that expression of these genes was upregulated after NAC, mostly in non-responsive patients (55–96 % of cases). Responsiveness to taxotere was related to reduced levels of *ABCB1*, *ABCC2, ABCG1, ABCG2*, and *MVP* mRNA in tumor samples collected after chemotherapy.
Conclusion Our results suggest that reductions in MDR gene expression in post-NAC samples in comparison with pre-NAC are associated with tumor response to FAC and CAX as well as taxotere-based NAC, while patients displaying MDR gene upregulation had resistance to therapy.

Keywords Multidrug resistance · Gene expression · Chemotherapy · Breast cancer

Electronic supplementary material The online version of this article (doi:10.1007/s00280-012-1992-x) contains supplementary material, which is available to authorized users.

N. V. Litviakov · N. V. Cherdyntseva (✉) · M. M. Tsyganov · E. V. Denisov · M. K. Merzliakova · V. V. Volkomorov
Department of Experimental Oncology, Cancer Research Institute, Siberian Branch of the Russian Academy of Medical Sciences, Tomsk 634050, Russian Federation
e-mail: nvch@oncology.tomsk.ru

N. V. Cherdyntseva · E. M. Slonimskaya
Department of Oncology, Siberian State Medical University, Tomsk, Russian Federation

E. Y. Garbukov · E. M. Slonimskaya
Department of General Oncology, Cancer Research Institute, Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russian Federation

S. V. Vtorushin · M. V. Zavyalova · V. M. Perelmuter
Department of Pathological Anatomy and Cytology, Cancer Research Institute, Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russian Federation

S. V. Vtorushin · M. V. Zavyalova · V. M. Perelmuter
Department of Pathological Anatomy, Siberian State Medical University, Tomsk, Russian Federation

Introduction

The development of multidrug resistance (MDR) followed by the failure of chemotherapy response is a critical problem in breast cancer (BC), but the underlying

molecular mechanisms are incompletely understood. Numerous studies have aimed to establish the role of ATP-dependent efflux pumps, also named ATP-binding cassette (ABC) transporters, in MDR and to link their expression to the chemotherapy response [1–5].

ABC transporters are encoded by a large family of genes, called MDR genes, of which the major ones are represented by the members of the *ABCB*, *ABCC*, and *ABCG* subfamilies [5]. Although a number of clinical studies have reported that a high level of tumor ABC transporters is associated with cancer progression, a clear link between expression level and chemotherapy sensitivity or disease outcome has not been identified [5]. Heterogeneity in the results is a common feature of studies evaluating associations between ABC transporter expression and response to chemotherapy, particularly neoadjuvant or preoperative therapy [3–6]; one of the main reasons for this is limitations of the assays that have been used [7].

Neoadjuvant chemotherapy is considered a suitable model for direct assessment of treatment response depending on MDR gene expression before and after therapy administration. Considering this fact, we focused on the key MDR genes (ABC transporters) expressed in breast tumors: *ABCB1* (MDR1), *ABCC1* (MRP1), *ABCC2* (MRP2), *ABCC3* (MRP3), *ABCC5* (MRP5), *ABCG1* (BCRP1), and *ABCG2* (BCRP), together with the glutathione S-transferase pi (*GSTP1*) gene, are involved in cellular phase II metabolism, detoxification and protection of tumor cells from genotoxic agents including chemotherapeutic drugs [8], and *MVP* (LRP1) operates as a cytoplasmic and/or nuclear membrane-associated drug transporter, perhaps in conjunction with ABC transporters [9]. The aim of this study was to investigate whether alterations in the levels of tumor expression of MDR genes (before and after NAC) in BC are associated with the response to NAC. Assessments of expression were carried out by TaqMan-based quantitative reverse transcriptase PCR (qRT-PCR), known as a superior method for MDR gene expression profiling [7]. It should be noted that in most studies mentioned above, MDR gene expression was evaluated just once before starting chemotherapy; however, it seems reasonable to assume that in clinical situations, not only the initial level of expression but also the active process of MDR formation (acquired or adaptive MDR) are of great significance.

Materials and methods

Patients and tumors

Patients ($n = 84$) with clinical stage IIA to IIIC ($T_{1\text{-}4}N_{0-3}M_0$) BC in the age range 28–61 years (mean age 46.95 ± 0.73) treated in the Cancer Research Institute (Tomsk, Russia) between 2006 and 2011 were included. The procedures followed in this study were in accordance with the Helsinki Declaration (1964, amended in 1975 and 1983). This study was approved by the institutional review board, and all patients signed an informed consent for voluntary participation. The histological diagnosis of invasive cancer, including estrogen (ER), progesterone (PR), and HER2 receptors were determined. The basic clinicopathological parameters of the patients are shown in Table 1.

All patients were primarily treated with NAC in accordance with "Consensus Conference on Neoadjuvant Chemotherapy (NAC) in Carcinoma of the Breast, April 26–28, 2003, Philadelphia, Pennsylvania" [10]. They received two to four preoperative cycles of FAC regimen (5-fluorouracil 600 mg/m^2, adriamycin 50 mg/m^2, and cyclophosphamide 600 mg/m^2 at intervals of 3 weeks), CAX (cyclophosphamide 100 mg/m^2 intramuscular injection, adriamycin 30 mg/m^2 intravenous injection, xeloda 1200 mg/m^2 oral administration), or taxotere in standard doses (100 mg/m^2 1-h infusion of 1 day) on the basis of body surface area, administered as a monotherapy.

Physical examination was performed before NAC and was repeated after 2 cycles of NAC and before surgery to determine clinical response. Imaging of the primary breast lesion was performed with mammography and/or ultrasonography. Clinical and imaging responses were categorized according to International Union Against Cancer criteria [11]. A complete response (CR) was defined as complete disappearance of primary tumor and lymph node metastasis. A partial response (PR) was determined as a tumor reduction >50 % and stable disease (SD) as a tumor reduction ≤50 % or a tumor size increase of <25 %. Progressive disease (PD) was described as an increase of >25 % in tumor size. In this way, patients were grouped into clinical responders (CR and PR) and non-responders (SD and PD).

Surgery (radical resection or sectoral resection or mastectomy) was performed within 1 to 2 weeks after the last administration of chemotherapy in responsive patients. After surgery, adjuvant chemotherapy or hormonal therapy was given.

Fresh BC tissues were obtained during the initial diagnostic biopsy (~10 mm^3) before NAC and in the course of tumor resection after NAC (~60–70 mm^3). The obtained tissue samples were stored in RNAlater solution (Ambion, USA) at −80 °C per the manufacturer's instructions until further use. Histological diagnosis was confirmed for all samples.

RNA isolation and cDNA synthesis

Total RNA was extracted from 84 samples of pre- and 75 post-NAC tumor tissues using the RNeasy mini kit plus

Table 1 The clinicopathological parameters of BC patients

Clinicopathological parameter	N (%)
Age (year)	
≤50	59 (70.2)
>50	25 (29.8)
Menstrual status	
Premenopausal	56 (66.7)
Postmenopausal	28 (33.3)
Histological type	
Invasive ductal carcinoma	71 (84.5)
Invasive lobular carcinoma	6 (7.1)
Medullary carcinoma	4 (4.8)
Others	3 (3.6)
Tumor size	
T_1	15 (17.9)
T_2	57 (67.9)
T_3	7 (8.3)
T_4	5 (5.9)
Lymph node metastasis	
N_0	37 (44.0)
N_1	34 (40.5)
N_2	10 (11.9)
N_3	3 (3.6)
Estrogen receptor	
Positive	37 (44.0)
Negative	30 (35.8)
No data	17 (20.2)
Progesterone receptor	
Positive	36 (42.9)
Negative	31 (36.9)
No data	17 (20.2)
HER2	
Negative	41 (48.8)
+	16 (19.1)
++	3 (3.6)
+++	6 (7.1)
No data	18 (21.4)
Histological form	
Unicentric	65 (77.4)
Multicentric	19 (22.6)
NAC regimen	
CAX	27 (32.1)
FAC	33 (39.3)
Taxotere	24 (28.6)
NAC response	
CR	9 (10.7)
PR	39 (46.4)
SD	27 (32.1)
PD	9 (10.7)

NAC neoadjuvant chemotherapy; *CAX* cyclophosphamide–adriamycin–xeloda, *FAC* 5-fluorouracil–adriamycin–cyclophosphamide, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, HER2 nuclear staining was observed in 10–50 % (+), 50–70 % (++), and 70–90 % (+++) of tumor cells, respectively

DNase I digestion (Qiagen, Germany); a total of 9 patients showed CR, rendering it impossible to obtain further tumor samples. Ribolock RNase inhibitor (Fermentas, Lithuania) was added to the isolated RNA.

RNA concentration and quality were measured with a NanoDrop-2000 spectrophotometer (Thermo Scientific, USA). The concentration of RNA ranged from 80 to 250 ng/μl. The optical density ratios at 260/280 and 260/230 to examine RNA quality were in the range of 1.95–2.05 and 1.90–2.31, respectively. RNA integrity was assessed by visualization of the 28S and 18S ribosomal RNA in 1.5 % agarose gels followed by 0.02 % ethidium bromide staining.

RNA was reverse transcribed into cDNA using the RevertAid kit with random hexanucleotide primers (Fermentas, Lithuania) following the manufacturer's instructions.

Quantitative real-time PCR

The expression levels of the *ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC5*, *ABCG1*, *ABCG2*, *GSTP1*, and *MVP* genes were measured by qRT-PCR based on TaqMan technology using a Rotor-Gene-6000 instrument (Corbett Research, Australia). In addition, one internal control gene—*GAPDH* (glyceraldehyde-3-phosphate dehydrogenase)—was used to normalize expression levels of the MDR genes.

qRT-PCR was performed in triplicate reactions in a volume of 15 μl containing 250 μM dNTPs (Sibenzyme, Russia), 300 nM forward and reverse primers, 200 nM probe, 2.5 mM $MgCl_2$, 1× SE buffer (67 mM Tris–HCl pH 8.8 at 25 °C, 16.6 mM $(NH_4)_2SO_4$, 0.01 % Tween-20), 2.5U Hot Start Taq polymerase (Sibenzyme, Russia), and 50 ng of template cDNA. Samples were heated for 10 min at 95 °C, followed by 40 cycles of amplification for 10 s at 95 °C and 20 s at 60 °C.

Primer and probe (FAM-BHQ1) sequences were designed using Vector NTI Advance 11.5, Oligo 7.5, and the NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore) (Table 2). Primers/probes were synthesized by the DNA-Synthesis Company (Russia). PCR products were visualized by 1.5 % agarose gel electrophoresis with 0.02 % ethidium bromide.

The mean expression level of each target gene was calculated for tumor tissue normalized to *GAPDH*. The average C_t (cycle threshold) was estimated for both the gene of interest and *GAPDH*. Relative expression was evaluated using the Pfaffl method, and the following formula was used to determine the expression ratio between the sample and the calibrator [12]:

Table 2 Sequence of the primers and probes used in the study

Genes	Amplicon (bp)	Sequence	Design
ABCB1 NM_000927.4	93	F 5′-gattgacagctacagcacgg-3′	OrD
		R 5′-ggtcgggtgggatagttga-3′	
		Probe 5′-tgccgaacacattggaaggaaa-3′	
ABCC1 NM_004996.3	87	F 5′-aggtgggctgcggaaag-3′	
		R 5′-cggagcccttgatagcca-3′	
		Probe 5′-tggctgagatggacaaagtggag-3′	
ABCC2 NM_000392.3	85	F 5′-cctgtcggctctgggaa-3′	
		R 5′-tgcccttgatggtgatgtg-3′	
		Probe 5′-ggactgctgtgggacatagg-3′	
ABCC3 NM_003786	68	F 5′-gcaccattgtcgtggctaca-3′	[13]
		R 5′-gcaggacacccaggaccat-3′	
		Probe 5′-catcctctcccacctgtccaagctca-3′	
ABCC5 NM_005688.2	76	F 5′-caagagggtaaactggttgga-3′	OrD
		R 5′-ctaaaatggctgaaatgagagag-3′	
		Probe 5′-ggcagtgtgggaagtggaaaa-3′	
ABCG1 NM_004915.3	78	F 5′-cctactacctggccaagaccat-3′	[14]
		R 5′-agtacacgatgctgcagtaggc-3′	
		Probe 5′-acgtgccctttcagatcatgttcccagt-3′	
ABCG2 NM_004827.2	97	F 5′-aaaggatgtctaagcaggga-3′	OrD
		R 5′-tgaggccaataaggtgagg-3′	
		Probe 5′-tcgaggctgatgaatggagaag-3′	
GAPDH NM_002046.3	92	F 5′-cacatcgctcagacaccat-3′	
		R 5′-gcaacaatatccactttaccaga-3′	
		Probe 5′-cgcccaatacgaccaaatccg-3′	
GSTP1 NM_000852.3	84	F 5′-ctggtggacatggtgaatgac-3′	
		R 5′-cttgcccgcctcatagttg-3′	
		Probe 5′-aggacctccgctgcaaatacatctc-3′	
MVP NM_017458.3	87	F 5′-ggaggtgctggaaaaggac-3′	
		R 5′-tcctcaaaatcaagcagcg-3′	
		Probe 5′-ctgcccaacactgccctccat-3′	

All Probes—FAM→BHQ1

NM number according to NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore), *bp* base pair, *F* forward primer, *R* reverse primer, *OrD* Original design

$$Ratio = \frac{(E_{target})^{\Delta Ct,target\ (calibrator-test)}}{(E_{ref})^{\Delta Ct,ref\ (calibrator-test)}}$$

The relative expression level was also normalized to a calibrator consisting of a pool of normal breast tissue specimens. For this purpose, specimens of adjacent normal breast tissue from 10 BC patients (NAC free) were used as a source of normal RNA. The results were articulated as n-fold differences in *ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC5*, *ABCG1*, *ABCG2*, *GSTP1*, and *MVP* gene expression relative to *GAPDH* and normal breast tissue.

Statistical analysis

Comparisons between qualitative attributes (e.g., ER+ and ER− or N_0 and N_{1-3}) were made using logistic regression, and the difference in expression level of each gene between the pre- and post-NAC condition was determined using the Wilcoxon signed-rank test. Relationships between changes in MDR gene expression in pre- and post-NAC samples, the clinicopathological characteristics of the patients, and different clinical responses to FAC, CAX, and taxotere chemotherapy were evaluated with the chi-square test. Statistical analyses were performed using STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA). Differences were significant if the p value was <0.05.

Results

According to clinical criteria, after NAC, 35/60 (58.3 %) of patients treated with FAC or CAX had objective tumor responses, whereas response failure was detected in 25/60 (41.7 %) patients. Patients who had been treated with

taxotere showed tumor responses in 13/24 (54.2 %) cases versus 11/24 (45.8 %) with no response. No significant association was found between tumor response and any clinicopathological parameter (Table 3).

To determine whether MDR gene expression correlated with patient and tumor characteristics, we screened pre-NAC samples of breast tumor from 84 patients using qRT-PCR (table in Online Resource 1). With one notable exception, no significant differences in expression level were observed according to patient age, menopausal status, tumor size, histological type (ductal or lobular), histological form (unicentric or multicentric), ER, PR, or nodal status. However, *ABCB1* expression appeared to be higher in T_{3-4} patients versus T_{1-2} patients ($p = 0.037$), while *ABCC5* and *MVP* levels depended on lymph node involvement ($p = 0.025$) and histological type ($p = 0.029$), respectively. The mean levels of expression of the studied genes before NAC did not differ between patients with responding or resistant tumors as shown in the table (Online Resource 1). In addition, no significant differences in average expression levels were observed between pre- and post-NAC samples when the total group was analyzed, including both responders and non-responders (data not shown).

We studied the expression of genes in paired pre- and post-operative breast tumor specimens ($n = 75$) and compared changes in their expression in response to NAC. Expression data were available for 75/84 tumors because post-operative tumor tissue from patients who had complete responses (9 patients) was not available to be screened. When alterations in gene expression between paired pre- and post-NAC specimens were calculated, they revealed cases showing either increases or decreases in expression level as well as some without expression. We

Table 3 Clinicopathological parameters and NAC response

Clinicopathological parameter	Response ($n = 48$)	Non-response ($n = 36$)	p value
Age (year)			
≤50	31	28	0.1906
>50	17	8	0.1906
Tumor size			
T_1	11	4	0.1621
T_2	30	27	0.2248
T_3	5	2	0.4250
T_4	2	4	0.2213
Lymph node metastasis			
N_0	20	17	0.6118
N_{1-3}	28	19	0.6118
Menopausal status			
Premenopausal	30	26	0.3496
Postmenopausal	18	10	0.3496
Histological type			
IDC	41	29	0.5541
ILC	2	4	0.2213
Others	5	3	0.7475
Histological form			
UC	38	25	0.1609
MC	8	11	0.1609
Estrogen receptor			
Positive	16	21	0.2745
Negative	17	13	0.2745
Progesterone receptor			
Positive	14	22	0.0674
Negative	19	12	0.0674
NAC regimen			
CAX	19	8	0.0918
FAC	16	17	0.1971
Taxotere	13	11	0.7274

Statistical analysis: p value—Chi-square (df = 1) test

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *UC* unicentric, *MC* multicentric, *NAC* neoadjuvant chemotherapy, *CAX* cyclophosphamide–adriamycin–xeloda, *FAC* 5-fluorouracil–adriamycin–cyclophosphamide

did not observe any association between clinicopathological parameters and numbers of patients showing increases or decreases in post-NAC expression of MDR genes. As an exception, we noted that expression of the *ABCB1* and *MVP* genes was frequently increased in unicentric versus multicentric patients ($p = 0.046$ and $p = 0.038$, respectively), while alterations in *ABCC3* activity depended on ER status ($p = 0.015$; table in Online Resource 2).

However, there was a significant correlation between tumor response to NAC and number of cases displaying expression changes in MDR genes after NAC. In the group of patients with different chemotherapy regimens, reductions in expression of MDR genes in post-NAC tumor samples compared with pre-NAC specimens were found in the majority of responders (63–90 % cases). On the contrary, gene upregulation was detected in 58–94 % of cases showing no objective response to chemotherapy ($10^{-13} < p < 0.02$; table in Online Resource 2). The mean values for gene expression in pre- and post-NAC tumor samples are presented for responders and non-responders in Fig. 1. The data illustrated here confirmed once again the results of table (Online Resource 2) and showed that expression level of the most studied genes, such as *ABCB1*, *ABCC1*, *ABCC2*, *ABCG1*, *ABCG2*, *MVP*, and *GSTP1,* was lower after NAC compared with before chemotherapy in responsive cases, whereas patients with resistance to chemotherapy displayed increases in the activities of the majority of the above-mentioned genes in post-chemotherapy tumor tissue in comparison with pre-NAC samples ($10^{-6} < p < 0.04$). Interestingly, the expression of *ABCG1* gene, detected as decreased in cases with response to NAC, did not significantly change between before and after chemotherapy in non-responsive subjects ($p = 0.20$; Fig. 1).

Alterations in gene expression following NAC were also assessed separately in the FAC-CAX and taxotere-treated patient groups. In the case of FAC-CAX regimens, comparison of pre- and post-NAC specimens showed that a high percentage of cases (from 67 to 93 %) with decreased expression of *ABCB1*, *ABCC1*, *ABCC2*, *ABCC5*, *ABCG1*, *ABCG2*, *MVP*, and *GSTP1* genes was in the group of responders, while non-responders (55–96 % of cases) displayed high expression of these genes after chemotherapy. In both responsive and resistant group, the high significance of differences was between cases with decrease and increase in expression of only *ABCB1*, *ABCC1*, *ABCC2*, and *ABCG2* genes ($10^{-9} < p < 10^{-5}$; Fig. 2). In taxotere, 85–92 % of responsive cases showed decrease in *ABCB1*, *ABCC2*, *ABCG1*, *ABCG2*, and *MVP* levels in post-NAC tumor samples relative to pre-chemotherapy specimens. Interestingly, 67–91 % of cases with response failure to taxotere had increased expression of the same genes ($10^{-5} < p < 0.03$; Fig. 2). In both the FAC-CAX- and taxotere-treated groups, the significant differences were shown between patients, which had alterations only in expression of *ABCB1*, *ABCC2,* and *ABCG2* genes during NAC (Fig. 2). Moreover, using logistic regression, we demonstrated strong associations between alterations in expression of these three genes and NAC response (OR = 78.9 [95 % CI: 14.87–508.27]; $p < 10^{-14}$).

Discussion

Neoadjuvant chemotherapy (NAC) is an integral part of a multimodality approach in the management of locally advanced BC [15], expanding surgical options, improving cosmetic results, and allowing oncologists to assess tumor response to therapy. Theoretically, it may also provide early control of micrometastatic disease [16, 17]. NAC is highly effective, with a clinical response rate ranging from 50 to 90 %, although with a much lower pathological complete response (pCR) rate, ranging from 2 to 27 % [18, 19]. The ability to achieve pCR is considered a key marker for adjuvant chemotherapy response and disease-free and overall survival [15, 16, 20].

It is clear that there are different molecular genetic markers associated with the presence or absence of pCR after pre-operative chemotherapy, which help tumors escape the toxicity induced by an ineffective chemotherapeutic regimen in non-responsive patients, and could assist planning an alternative course of therapy. Currently, clinical tests for predicting cancer response are not available, and individual markers have shown little predictive value [21, 22]; however, the latest studies show great promise in developing tests to predict chemotherapy response [23–25].

It is well established that many breast tumors that initially respond to chemotherapy subsequently develop resistance to a broad range of drugs. In most cases, drug resistance is the outcome of a variety of cellular and pharmacological processes, of which the most significant is the activity of ABC transporters ejecting cytostatic agents from tumor cells against the concentration gradient with ATP energy consumption [5]. In the present study, we have focused on the key MDR genes that encode ABC transporters: *ABCB1* (MDR1), *ABCC1* (MRP1), *ABCC2* (MRP2), *ABCC3* (MRP3), *ABCC5* (MRP5), *ABCG1* (BCRP1), and *ABCG2* (BCRP), the increased expression of which may provoke resistance to neoadjuvant chemotherapy in BC. In addition, taking into account the major role of *GSTP1* in metabolite detoxification [8] and *MVP* (LRP1), which acts as a cytoplasmic and/or nuclear membrane-associated drug transporter, perhaps in conjunction with ABC transporters [9], these genes have also been included in our study.

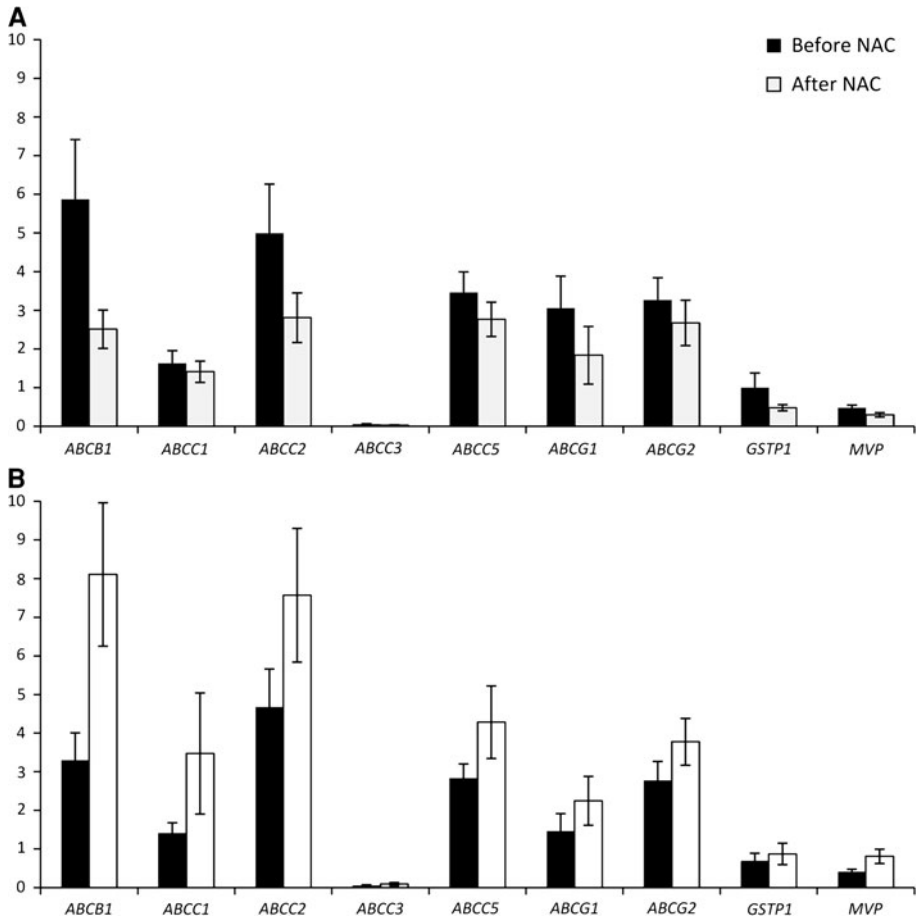

Fig. 1 The expression level of MDR genes in breast tumors collected before and after neoadjuvant chemotherapy (NAC). The expression level, as mean and standard error (M ± SE), was given for patients with partial response (**a**, $n = 38$) and stabilization/progression of disease (**b**, $n = 27$) after NAC. Statistical analysis: p value—Wilcoxon signed ranks test. Significant differences were shown for *ABCB1* ($p = 5.0 \times 10^{-6}$), *ABCC1* (0.0349), *ABCC2* (8.0×10^{-6}), *ABCG1* (7.8×10^{-5}), *ABCG2* (4.9×10^{-5}), *GSTP1* (0.0018), and *MVP* (0.0003) genes in the group with partial response and for *ABCB1* (2.0×10^{-6}), *ABCC1* (0.0179), *ABCC2* (0.0007), *ABCG2* (5.0×10^{-5}), *GSTP1* (0.0179), and *MVP* (0.0376) genes in the group with stabilization/progression of disease

The role played by MDR genes in clinical treatment is a subject of debate. A number of studies have attempted to assess the relationship between MDR gene expression and type of chemotherapy response as well as disease outcome in BC patients; however, there are many contradictions in the data [3–6]. In the present study, we did not demonstrate a link between the pre-NAC levels of MDR gene expression with any clinicopathological parameter of BC except some casual observations (table in Online Resource 1). Our observations, which are in accordance with previous data [26–29], most likely explain the weak association of clinicopathological parameters with NAC response in BC.

In most studies, MDR gene expression was evaluated once before starting chemotherapy; however, it is known that chemotherapy may modify gene expression, and it seems reasonable to assume that not only the initial level of expression but also the active process of MDR formation are of great significance. Based on the above reasoning, we analyzed whether chemotherapy induced changes in MDR gene expression correlate with immediate clinical response to NAC. The expression of the MDR genes listed above was evaluated in paired breast tumor samples collected before and after NAC. In our study, MDR gene activity was increased in non-responsive patients and decreased in responders after NAC. These data are in agreement with results of Linn et al. [30] reporting changes in expression of *ABCC1*, *ABCB1*, and MVP genes in opposing directions in NAC, and observations of Chevillard and colleagues [31] demonstrating an association between up- and downregulation of *ABCB1* gene expression after chemotherapy with resistance and sensitivity to NAC, respectively. A more recent study [32] also showed that BC patients in whom expression of the *ABCB1* gene was induced during the process of NAC did not respond to chemotherapy, whereas cases without gene upregulation displayed high rates of successful treatment.

In accordance with the above-mentioned studies and other studies [33–36], the changing expression of MDR genes during NAC is a well-known phenomenon; however, there is heterogeneity in establishing a clear link between a unidirectional change in MDR gene activity (increase/decrease) with chemotherapy efficiency. In particular, Singh and coauthors [35] demonstrated increase in *ABCB1* gene expression after NAC in both responsive and non-responsive BC cases. Faneyte et al. [33, 34] also failed to show significant differences in post-NAC expression levels of *ABCB1* and *ABCC1-3* in responders and non-responders with BC.

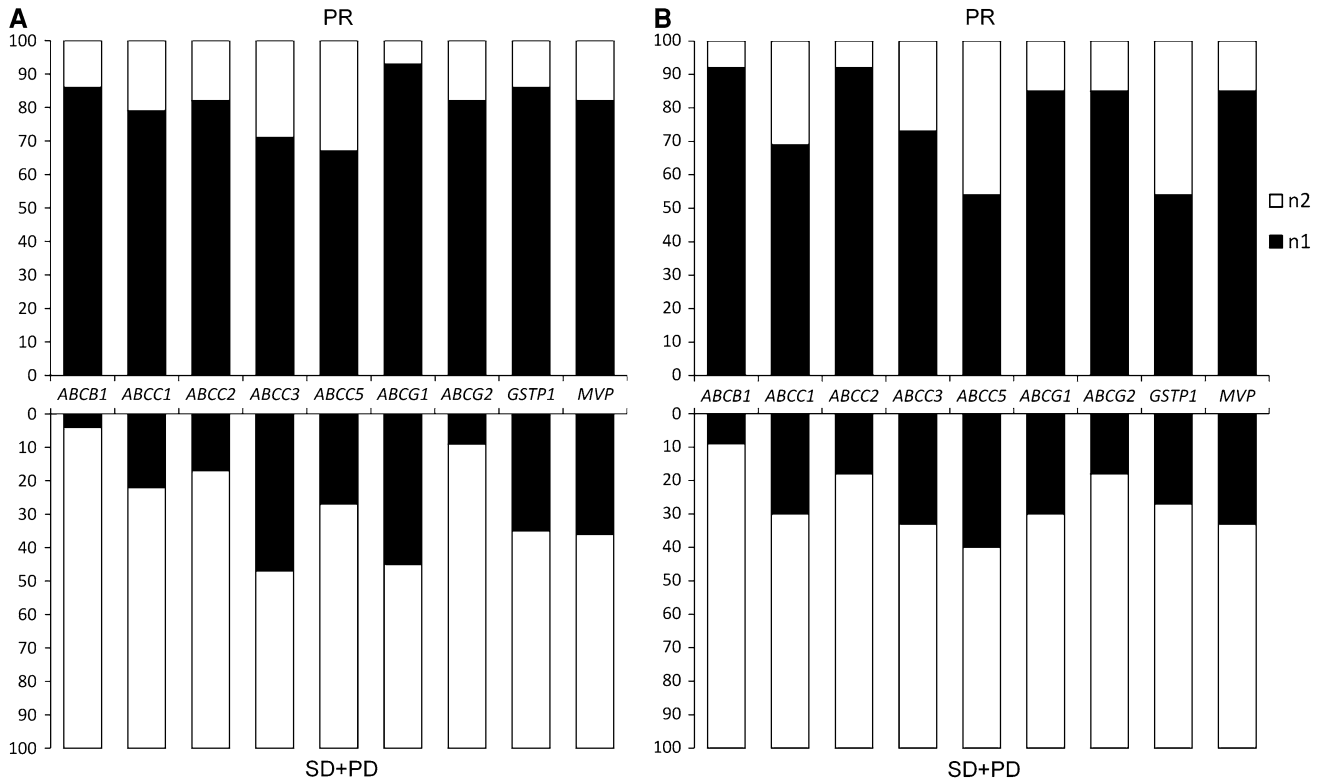

Fig. 2 The percentage of cases with alteration in MDR gene expression depending on different neoadjuvant chemotherapy (NAC) regimen and response. Patients treated in cyclophosphamide–adriamycin–xeloda (CAX) and 5-fluorouracil–adriamycin–cyclophosphamide (FAC) regimens are presented in Fig. 2a, patients with taxotere—in Fig. 2b. PR designates partial response, *SD* stable disease, *PD* progressive disease. n1 means the number of patients with decrease in gene expression or without expression after neoadjuvant chemotherapy, n2—the number of patients with increase in gene expression after neoadjuvant chemotherapy. Statistical analysis: p value—Chi-squared (df = 1) test. Significant differences were shown for *ABCB1* ($p = 2.2 \times 10^{-9}$), *ABCC1* (6.9×10^{-5}), *ABCC2* (4.4×10^{-6}), *ABCC5* (0.0096), *ABCG1* (0.0003), *ABCG2* (1.2×10^{-7}), *GSTP1* (0.0003), and *MVP* (0.0013) genes in the group with CAX-FAC and for *ABCB1* (8.9×10^{-5}), *ABCC2* (0.0006), *ABCG1* (0.0131), *ABCG2* (0.0031), and *MVP* (0.0260) genes in the group with taxotere

The alterations of MDR gene activity during NAC is a well-known phenomenon related to regulation of their expression and/or change in the total number of tumor cells expressing them, as has been shown by immunohistochemistry more than once [2]. Genetic elements and processes such as DNA methylation, histone deacetylation, transacting proteins (transcription factors), DNA sequence variants, and microRNAs (miRNAs) could be involved in the modulation of MDR gene expression at either the transcriptional or translational levels [37–39].

A reduction in MDR gene activity during NAC could be the result of expression inhibition and/or a lower percentage of tumor cells expressing the genes owing to cell death during chemotherapy [2, 34]. Our data indicate that along with the influence of reduction of number of tumor cells, a decrease in MDR gene expression is possible caused by strong repression of transcription during chemotherapy. In particular, the study of level of MDR genes in biopsy samples, taken from four patients before NAC and after 7 days from start of CAX chemotherapy, showed a twofold decrease in expression in 3/4 (75.0 %) patients, although therapy response was still non-significant. Further, all these patients displayed partial regression of tumor (own unpublished data). The well-known fact in the regulation of MDR gene transcription is strong binding promoter of the *ABCB1* gene, one of the most important drug transporters, by the wild-type p53 protein and further repression of transcription [40, 41]. It is not excluded that p53, high level of which is induced by chemotherapeutic agents due to DNA damage, can downregulate *ABCB1* transcription. However, mutation analysis is needed to clarify p53 status, because it is known that mutant p53 upregulates *ABCB1* expression [42]. In addition, because surgery was performed one to 2 weeks after the end of NAC, MDR gene expression in post-NAC tumor samples could be restored to normal by natural processes.

An increase in MDR gene activity after NAC is evidently related with expression induction and/or selection of resistant clones with high expression of drug transporters and further increase in their number. So, there are many

data demonstrating that chemotherapy agents decrease DNA methylation and inhibit activity of histone deacetylases [43–45] that can make MDR gene promoters accessible and competent for subsequent transcriptional activation. Different mechanisms of induction of DNA hypomethylation by chemotherapeutic drugs have been described in the recent review [45]. It is also known that deregulation of Raf/MEK/ERK, MAPK, and JNK pathways, which could be caused by chemotherapy, induces the expression of drug transporters [46, 47]. Again, it should not be forgotten that mutant p53 induces MDR gene expression, as it was already mentioned above.

It must be taken into account that in many cases, the expression of MDR genes is inversely correlated with the levels of several miRNAs. In turn, miRNA activity is greatly modulated by chemotherapeutic drugs most likely via DNA damage, DNA demethylation, and histone deacetylase inhibition, which were found to be related to extensive and rapid alteration of microRNA levels [48, 49].

Therefore, in clinical practice, as opposed to in vitro, there is a chemotherapy-linked process of MDR formation controlled both by the organism and by the tumor factors. The study of MDR development appears to be a key factor for understanding mechanisms of chemoresistance. Therefore, to predict chemotherapy efficiency, it is necessary to understand why, with the same treatment regimen, some patients display a decrease in MDR gene expression and a good response, while others display gene upregulation and resistance to therapy. The answer to the question most likely lies in individual features of the patients and the tumor. Aside from the chemotherapy-related factors modifying MDR gene expression, gene polymorphism is one of the major keys to understanding the development of MDR gene expression and drug resistance in individual patients, a point that was partially demonstrated in our previous papers [50, 51]. It should be noted that intratumoral morphological heterogeneity in BC or the presence of five different morphological types of infiltrating components (morphological structures), mirroring the architectural arrangements of tumor cells [52], results in diverse "portrait" of MDR gene expression [53] and different response to NAC [54] within one tumor.

Taken together, the data in this study suggest that changes in the expression vector of *ABCB1* (*MDR1*), *ABCC1* (*MRP1*), *ABCC2* (*MRP2*), *ABCC3* (MRP3), *ABCC5* (*MRP5*), *ABCG1* (*BCRP1*), and *ABCG2* (*BCRP*) genes during the chemotherapy process or the development of adaptive MDR, but not the mRNA levels of these genes per se, are associated with NAC efficiency. Increases in MDR gene expression after NAC resulted in poor responses, whereas decreases were related to high chemotherapy efficiency. Once again, we would like to note that future studies should focus on the molecular basis of how the expression of these transporters is regulated in normal breast cells and in their malignant counterparts, as previously suggested by Prof. M. Tien Kuo. These studies may lead to novel strategies of controlling MDR through gene regulation [4].

Acknowledgments This work was supported by the Federal Target Program "Academic and Teaching Staff of Innovative Russia in 2009–2013" (#16.740.11.0606 and #8291) of the Ministry of Education and Science of the Russian Federation, the Russian Federation President Grant (#MK-1259.2012.7), and a grant from the OPTEK company (#1/11KTS).

Conflict of interest The other authors have no conflicts of interest.

References

1. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM (2006) Targeting multidrug resistance in cancer. Nat Rev Drug Discov 5(3):219–234. doi:10.1038/nrd1984
2. Leonessa F, Clarke R (2003) ATP binding cassette transporters and drug resistance in breast cancer. Endocr Relat Cancer 10(1):43–73
3. Fletcher JI, Haber M, Henderson MJ, Norris MD (2010) ABC transporters in cancer: more than just drug efflux pumps. Nat Rev Cancer 10(2):147–156. doi:10.1038/nrc2789
4. Kuo MT (2007) Roles of multidrug resistance genes in breast cancer chemoresistance. Adv Exp Med Biol 608:23–30
5. Wind N, Holen I (2011) Multidrug resistance in breast cancer–from in vitro models to clinical studies. Int J Breast Cancer 2011:1–12. doi:10.4061/2011/967419
6. Gillet JP, Efferth T, Remacle J (2007) Chemotherapy-induced resistance by ATP-binding cassette transporter genes. Biochim Biophys Acta 1775(2):237–262. doi:10.1016/j.bbcan.2007.05.002
7. Gillet JP, Gottesman MM (2011) Advances in the molecular detection of ABC transporters involved in multidrug resistance in cancer. Curr Pharm Biotechnol 12(4):686–692
8. Vasieva O (2011) The many faces of glutathione transferase pi. Curr Mol Med 11(2):129–139
9. Mossink MH, van Zon A, Scheper RJ, Sonneveld P, Wiemer EA (2003) Vaults: a ribonucleoprotein particle involved in drug resistance? Oncogene 22(47):7458–7467. doi:10.1038/sj.onc.1206947
10. Schwartz GF, Hortobagyi GN (2004) Proceedings of the consensus conference on neoadjuvant chemotherapy in carcinoma of the breast, April 26–28, 2003, Philadelphia, Pennsylvania. Cancer 100(12):2512–2532. doi:10.1002/cncr.20298
11. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD (1977) Assessment of response to therapy in advanced breast cancer: a project of the programme on clinical oncology of the International union against cancer, Geneva. Switz Cancer 39(3):1289–1294
12. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29(9):e45
13. Steinbach D, Lengemann J, Voigt A, Hermann J, Zintl F, Sauerbrey A (2003) Response to chemotherapy and expression of the genes encoding the multidrug resistance-associated proteins MRP2, MRP3, MRP4, MRP5, and SMRP in childhood acute myeloid leukemia. Clin Cancer Res 9(3):1083–1086
14. Nishimura M, Yoshitsugu H, Naito S, Hiraoka I (2002) Evaluation of gene induction of drug-metabolizing enzymes and transporters in primary culture of human hepatocytes using high-sensitivity real-time reverse transcription PCR. Yakugaku Zasshi 122(5):339–361

15. Gralow JR, Burstein HJ, Wood W, Hortobagyi GN, Gianni L, von Minckwitz G, Buzdar AU, Smith IE, Symmans WF, Singh B, Winer EP (2008) Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. J Clin Oncol 26(5):814–819. doi:10.1200/JCO.2007.15.3510
16. Caudle AS, Gonzalez-Angulo AM, Hunt KK, Liu P, Pusztai L, Symmans WF, Kuerer HM, Mittendorf EA, Hortobagyi GN, Meric-Bernstam F (2010) Predictors of tumor progression during neoadjuvant chemotherapy in breast cancer. J Clin Oncol 28(11):1821–1828. doi:10.1200/JCO.2009.25.3286
17. Precht LM, Lowe KA, Atwood M, Beatty JD (2010) Neoadjuvant chemotherapy of breast cancer: tumor markers as predictors of pathologic response, recurrence, and survival. Breast J 16(4): 362–368. doi:10.1111/j.1524-4741.2010.00935.x
18. Alba E, Calvo L, Albanell J, De la Haba JR, Arcusa Lanza A, Chacon JI, Sanchez-Rovira P, Plazaola A, Lopez Garcia-Asenjo JA, Bermejo B, Carrasco E, Lluch A (2012) Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. Ann Oncol. doi:10.1093/annonc/mds132
19. Berruti A, Brizzi MP, Generali D, Ardine M, Dogliotti L, Bruzzi P, Bottini A (2008) Presurgical systemic treatment of nonmetastatic breast cancer: facts and open questions. Oncologist 13(11):1137–1148. doi:10.1634/theoncologist.2008-0162
20. Kaufmann M, von Minckwitz G, Smith R, Valero V, Gianni L, Eiermann W, Howell A, Costa SD, Beuzeboc P, Untch M, Blohmer JU, Sinn HP, Sittek R, Souchon R, Tulusan AH, Volm T, Senn HJ (2003) International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer: review and recommendations. J Clin Oncol 21(13):2600–2608. doi:10.1200/JCO.2003.01.136
21. Fumagalli D, Desmedt C, Ignatiadis M, Loi S, Piccart M (2011) Sotiriou C (2011) Gene profiling assay and application: the predictive role in primary therapy. J Natl Cancer Inst Monogr 43:124–127. doi:10.1093/jncimonographs/lgr040
22. Nagasaki K, Miki Y (2008) Molecular prediction of the therapeutic response to neoadjuvant chemotherapy in breast cancer. Breast Cancer 15(2):117–120. doi:10.1007/s12282-008-0031-6
23. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Graf S, Ha G, Haffari G, Bashashati A, Russell R, McKinney S, Caldas C, Aparicio S, Brenton JD, Ellis I, Huntsman D, Pinder S, Purushotham A, Murphy L, Bardwell H, Ding Z, Jones L, Liu B, Papatheodorou I, Sammut SJ, Wishart G, Chia S, Gelmon K, Speers C, Watson P, Blamey R, Green A, Macmillan D, Rakha E, Gillett C, Grigoriadis A, di Rinaldis E, Tutt A, Parisien M, Troup S, Chan D, Fielding C, Maia AT, McGuire S, Osborne M, Sayalero SM, Spiteri I, Hadfield J, Bell L, Chow K, Gale N, Kovalik M, Ng Y, Prentice L, Tavare S, Markowetz F, Langerod A, Provenzano E, Borresen-Dale AL (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486:346–352. doi:10.1038/nature10983
24. Balko JM, Cook RS, Vaught DB, Kuba MG, Miller TW, Bhola NE, Sanders ME, Granja-Ingram NM, Smith JJ, Meszoely IM, Salter J, Dowsett M, Stemke-Hale K, Gonzalez-Angulo AM, Mills GB, Pinto JA, Gomez HL, Arteaga CL (2012) Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. Nat Med 18:1052–1059. doi:10.1038/nm.2795
25. Hatzis C, Pusztai L, Valero V, Booser DJ, Esserman L, Lluch A, Vidaurre T, Holmes F, Souchon E, Wang H, Martin M, Cotrina J, Gomez H, Hubbard R, Chacon JI, Ferrer-Lozano J, Dyer R, Buxton M, Gong Y, Wu Y, Ibrahim N, Andreopoulou E, Ueno NT, Hunt K, Yang W, Nazario A, DeMichele A, O'Shaughnessy J, Hortobagyi GN, Symmans WF (2011) A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. JAMA 305(18):1873–1881. doi:10.1001/jama.2011.593
26. Keskin S, Muslumanoglu M, Saip P, Karanlik H, Guveli M, Pehlivan E, Aydogan F, Eralp Y, Aydiner A, Yavuz E, Ozmen V, Igci A, Topuz E (2011) Clinical and pathological features of breast cancer associated with the pathological complete response to anthracycline-based neoadjuvant chemotherapy. Oncology 81(1):30–38. doi:10.1159/000330766
27. Kaufmann M, von Minckwitz G, Mamounas EP, Cameron D, Carey LA, Cristofanilli M, Denkert C, Eiermann W, Gnant M, Harris JR, Karn T, Liedtke C, Mauri D, Rouzier R, Ruckhaeberle E, Semiglazov V, Symmans WF, Tutt A, Pusztai L (2012) Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol 19(5):1508–1516. doi:10.1245/s10434-011-2108-2
28. Lacave R, Coulet F, Ricci S, Touboul E, Flahault A, Rateau JG, Cesari D, Lefranc JP, Bernaudin JF (1998) Comparative evaluation by semiquantitative reverse transcriptase polymerase chain reaction of MDR1, MRP and GSTp gene expression in breast carcinomas. Br J Cancer 77(5):694–702
29. Moureau-Zabotto L, Ricci S, Lefranc JP, Coulet F, Genestie C, Antoine M, Uzan S, Lotz JP, Touboul E, Lacave R (2006) Prognostic impact of multidrug resistance gene expression on the management of breast cancer in the context of adjuvant therapy based on a series of 171 patients. Br J Cancer 94(4):473–480. doi:10.1038/sj.bjc.6602958
30. Linn SC, Pinedo HM, van Ark-Otte J, van der Valk P, Hoekman K, Honkoop AH, Vermorken JB, Giaccone G (1997) Expression of drug resistance proteins in breast cancer, in relation to chemotherapy. Int J Cancer 71(5):787–795
31. Chevillard S, Pouillart P, Beldjord C, Asselain B, Beuzeboc P, Magdelenat H, Vielh P (1996) Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. Cancer 77(2):292–300. doi:10.1002/(SICI)1097-0142(19960115)77:2<292:AID-CNCR11>3.0.CO;2-X
32. Atalay C, Deliloglu Gurhan I, Irkkan C, Gunduz U (2006) Multidrug resistance in locally advanced breast cancer. Tumour Biol 27(6):309–318. doi:10.1159/000096086
33. Faneyte IF, Kristel PM, van de Vijver MJ (2004) Multidrug resistance associated genes MRP1, MRP2 and MRP3 in primary and anthracycline exposed breast cancer. Anticancer Res 24(5A): 2931–2939
34. Faneyte IF, Kristel PM, van de Vijver MJ (2001) Determining MDR1/P-glycoprotein expression in breast cancer. Int J Cancer 93(1):114–122. doi:10.1002/1097-0215(20010701)93:1<114:AID-IJC1309>3.0.CO;2-J
35. Singh LC, Chakraborty A, Mishra AK, Devi TR, Sugandhi N, Chintamani C, Bhatnagar D, Kapur S, Saxena S (2012) Study on predictive role of AR and EGFR family genes with response to neoadjuvant chemotherapy in locally advanced breast cancer in Indian women. Med Oncol 29(2):539–546. doi:10.1007/s12032-011-9952-6
36. Gillet JP, Efferth T, Steinbach D, Hamels J, de Longueville F, Bertholet V, Remacle J (2004) Microarray-based detection of multidrug resistance in human tumor cells by expression profiling of ATP-binding cassette transporter genes. Cancer Res 64(24):8987–8993. doi:10.1158/0008-5472.CAN-04-1978
37. Glubb DM, Innocenti F (2011) Mechanisms of genetic regulation in gene expression: examples from drug metabolizing enzymes and transporters. WIREs Syst Biol Med 3:299–313. doi:10.1002/wsbm.125
38. Sharma G, Mirza S, Parshad R, Srivastava A, Datta Gupta S, Pandya P, Ralhan R (2010) CpG hypomethylation of MDR1 gene

in tumor and serum of invasive ductal breast carcinoma patients. Clin Biochem 43(4–5):373–379. doi:10.1016/j.clinbiochem.2009.10.009
39. Reed K, Parissenti AM (2010) Epigenetic regulation of ABCB1 transporter expression and function. Curr Pharmacol 8(3):218–231
40. Johnson RA, Ince TA, Scotto KW (2001) Transcriptional repression by p53 through direct binding to a novel DNA element. J Biol Chem 276(29):27716–27720. doi:10.1074/jbc.C100121200
41. Thottassery JV, Zambetti GP, Arimori K, Schuetz EG, Schuetz JD (1997) p53-dependent regulation of MDR1 gene expression causes selective resistance to chemotherapeutic agents. Proc Natl Acad Sci USA 94(20):11037–11042
42. Sampath J, Sun D, Kidd VJ, Grenet J, Gandhi A, Shapiro LH, Wang Q, Zambetti GP, Schuetz JD (2001) Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. J Biol Chem 276(42):39359–39367. doi:10.1074/jbc.M103429200
43. Hanafy S, Salem T, El-Aziz A, EL-Fiky B, Shokair M (2011) Influence of anticancer drugs on DNA methylation in liver of female mice. Am J Mol Biol 1:62–69. doi:0.4236/ajmb.2011.12008
44. Samlowski WE, Leachman SA, Wade M, Cassidy P, Porter-Gill P, Busby L, Wheeler R, Boucher K, Fitzpatrick F, Jones DA, Karpf AR (2005) Evaluation of a 7-day continuous intravenous infusion of decitabine: inhibition of promoter-specific and global genomic DNA methylation. J Clin Oncol 23(17):3897–3905. doi:10.1200/JCO.2005.06.118
45. Wilting RH, Dannenberg JH (2012) Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. Drug Resist Updat 15:21–38. doi:10.1016/j.drup.2012.01.008
46. Sui H, Fan ZZ, Li Q (2012) Signal transduction pathways and transcriptional mechanisms of ABCB1/Pgp-mediated multiple drug resistance in human cancer cells. J Int Med Res 40(2):426–435
47. Weinstein-Oppenheimer CR, Henriquez-Roldan CF, Davis JM, Navolanic PM, Saleh OA, Steelman LS, Franklin RA, Robinson PJ, McMahon M, McCubrey JA (2001) Role of the Raf signal transduction cascade in the in vitro resistance to the anticancer drug doxorubicin. Clin Cancer Res 7(9):2898–2907
48. Iorio MV, Croce CM (2012) microRNA involvement in human cancer. Carcinogenesis. doi:10.1093/carcin/bgs140
49. Hu H, Gatti RA (2011) MicroRNAs: new players in the DNA damage response. J Mol Cell Biol 3(3):151–158. doi:10.1093/jmcb/mjq042
50. Litviakov NV, Cherdyntseva NV, Tsyganov MM, Denisov EV, Merzliakova MK, Garbukov EY, Vtorushin SV, Zavyalova MV, Slonimskaya EM (2011) Influence of gene polymorphism on the expression of the multidrug resistance genes in breast tumor during neoadjuvant chemotherapy. Med Genet (Russ) 10(10):37–43
51. Gervas PA, Litviakov NV, Stakcheeva MN, Miller SV, Dobrodeev AY, Garbukov EY, Babyshkina NN, Kukharev YV, Vasilieva MV, Slonimskaya EM, Belyavskaya VA, Tuzikov SA, Cherdyntseva NV (2009) Influence of apoptosis and repair gene polymorphism on neoadjuvant chemotherapy response of malignant tumors. Sib J Oncol (Russ) 4(34):41–47
52. Zavyalova MV, Perelmuter VM, Vtorushin SV, Denisov EV, Litvyakov NV, Slonimskaya EM, Cherdyntseva NV (2011) The presence of alveolar structures in invasive ductal NOS breast carcinoma is associated with lymph node metastasis. Diagn Cytopathol. doi:10.1002/dc.21852
53. Denisov E, Tsyganov M, Tashireva L, Zavyalova M, Perelmuter V, Cherdyntseva N (2012) Intratumoral heterogeneity in expression of chemotherapy response markers in invasive ductal breast carcinoma NOS. 4th WIN Symposium: efficacy of biomarkers and personalized cancer therapeutics, Paris, France. Ann Oncol 23(5):v21–v22
54. Zavyalova MV, Litvyakov NV, Garbukov EY, Vtorushin SV, Stakheeva MN, Savenkova OV, Kritskaya NG, Perelmuter VM, Slonimskaya EM, Cherdyntseva NV (2008) Relationship between tumor sensitivity to neoadjuvant chemotherapy and histologic pattern of primary tumor in unicentric infiltrating ductal breast carcinoma. Sib J Oncol (Russ) 6:30–34