*Int. J. Cancer (Pred. Oncol.):* **95,** 255–259 (2001)


Publication of the International Union Against Cancer

# DECREASED EXPRESSION OF *BRCA2* mRNA PREDICTS FAVORABLE RESPONSE TO DOCETAXEL IN BREAST CANCER

Chiyomi EGAWA, Yasuo MIYOSHI, Yuuki TAKAMURA, Tetsuya TAGUCHI, Yasuhiro TAMAKI and Shinzaburo NOGUCHI*

*Department of Surgical Oncology, Osaka University Medical School, Osaka, Japan*

**The clinical usefulness of *BRCA1* and *BRCA2* mRNA levels in tumor tissues in the prediction of response to docetaxel (DOC) treatment has been studied in breast-cancer patients. Twenty-five patients with locally advanced breast tumors (n = 13) or locally recurrent tumors (n = 12) underwent tumor biopsy and were treated with DOC (60 mg/m$^2$ every 3 weeks). *BRCA1* and *BRCA2* mRNA levels in the tumors were determined by real-time PCR, and the expression of 6 biological markers (P-glycoprotein, p53, erbB2, BCL2, MIB1, estrogen receptor-α) in the tumors was determined by immunohistochemistry. *BRCA2* mRNA levels (0.547 ± 0.200, mean ± SE) of responders to DOC treatment were significantly ($p < 0.05$) lower than those of non-responders (1.538 ± 0.358), but there was no significant difference in *BRCA1* mRNA levels between responders (0.389 ± 0.081) and non-responders (0.779 ± 0.172). Tumors were dichotomized into groups with high or low *BRCA2* mRNA levels according to the cut-off value of 0.13. The response rate (25%) of tumors with high *BRCA2* mRNA levels was significantly ($p < 0.01$) lower than that (100%) of tumors with low *BRCA2* mRNA levels. Positive predictive value, negative predictive value and diagnostic accuracy of the *BRCA2* mRNA assay in the prediction of response to DOC were 100%, 75% and 80%, respectively. No significant difference was found between responders and non-responders in the expression status of any of the other 6 biological markers. These results suggest that *BRCA2* mRNA levels in tumor tissues might be clinically useful in the prediction of response to DOC treatment in breast-cancer patients.**


**Key words:** *breast cancer; BRCA2; BRCA1; docetaxel; prediction*

*BRCA1* and *BRCA2* are well-established breast cancer–susceptibility genes and have been cloned through linkage analysis using large breast-cancer families.[1,2] Both are considered to be classical tumor-suppressor genes since loss of both alleles is required for carcinogenesis.[3,4] Several important functions of *BRCA1* and *BRCA2* have been disclosed,[5] including regulation of the $G_2$–M checkpoint. *BRCA1* and *BRCA2* co-localize to centrosomes during mitosis and control the assembly of mitotic spindles as well as the appropriate segregation of chromosomes to daughter cells.[6,7] Mouse fibroblasts carrying a mutant *Brca1* are unable to arrest at $G_2$–M and suffer from amplification of the centrosomes, resulting in abnormal chromosomal segregation and aneuploidy.[8] Consistent with this *in vitro* finding, we have shown that low *BRCA1* expression is significantly associated with chromosomal instability in human breast cancer.[9]

Somatic mutation of *BRCA1* and *BRCA2* is very rare,[10–13] but loss of heterozygosity is frequently observed at 17q12-q21 and 13q12-q13, implicating these 2 genes in the pathogenesis of breast cancer through a regulatory (not a structural) mutation or through hypermethylation of the promoter region leading to attenuated transcription. *BRCA1* mRNA levels are mostly down-regulated and *BRCA2* mRNA levels are both up-regulated and down-regulated in sporadic breast cancers compared with normal breast tissue.[14–16] Hypermethylation of the promoter region of *BRCA1* explains the down-regulation in some sporadic breast cancers.[14,17] Since the promoter region of *BRCA2*, unlike *BRCA1*, is not hypermethylated,[18] another mechanism appears to be operative in the down-regulation of *BRCA2* mRNA.

Docetaxel (DOC) is one of the most active anti-neoplastic drugs in the treatment of breast cancer.[19] DOC binds to and stabilizes microtubules.[20] Mitotic spindles are the microtubules most sensitive to DOC treatment; thus, DOC affects the assembly of mitotic spindles to the centrosomes and induces cell-cycle arrest at $G_2$–M phase, culminating in apoptosis.[20] Since *BRCA1* and *BRCA2* also play an important role in the assembly of mitotic spindles, it is speculated that tumors with low expression of *BRCA1* and *BRCA2* mRNA might be more sensitive to DOC treatment.

It is important to develop predictors of response to DOC treatment, to improve treatment efficiency. Although several mechanisms of resistance to DOC have been postulated experimentally,[19] no predictors with clinical usefulness have been demonstrated in breast cancer. Thus, in the present study, we studied the clinical usefulness of *BRCA1* and *BRCA2* mRNA expression in tumor tissues as predictors of response to DOC treatment in breast-cancer patients. In addition, the clinical usefulness of various other biological markers [P-glycoprotein (P-gp), p53, erbB2, BCL2, MIB1, estrogen receptor (ER)], often studied as predictors of doxorubicin treatment, was also studied.

## MATERIAL AND METHODS

### *Patients*

Twenty-five female patients (median age 51 years, range 34–65) with locally advanced breast cancer (n = 13) or locally recurrent breast cancer in the chest wall (n = 7) or in the regional lymph node (n = 5) were recruited. Patient characteristics are shown in Table I. Of the 12 patients with locally recurrent tumors, 1 had lung metastases and 4 had bone metastases concurrently. All patients underwent biopsy of breast tumors or locally recurrent tumors (incisional biopsy or vacuum-associated core needle biopsy) before chemotherapy. Surgical specimens were snap-frozen in liquid nitrogen and kept at −80°C until use.

Four cycles of DOC (60 mg/m$^2$ i.v. every 3 weeks) were given to patients with locally advanced breast tumors as neoadjuvant chemotherapy, and DOC was given until disease progression to patients with locally recurrent tumors. Chemotherapy and hormonotherapy prior to DOC treatment in patients with locally recurrent tumors are described in Table II.

### *RNA extraction*

Total cellular RNA was extracted from surgical specimens using TRIZOL reagent according to the protocol provided by the manufacturer (Molecular Research Center, Cincinnati, OH). The 3 μg of total RNA were reverse-transcribed for single-strand cDNA using oligo-(dT)15 primer and Superscript II (Life Technologies, Gaithersburg, MD) and scaled up to a final volume of 50 μl. The RT reaction was performed at 42°C for 90 min, followed by heating at 70°C for 10 min.

Grant sponsor: Ministry of Education, Science, Sports and Culture, Japan.

*Correspondence to: Department of Surgical Oncology, Osaka University Medical School, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan. Fax: +81-06-6879-3779. E-mail: noguchi@onsurg.med.osaka-u.ac.jp

Received 29 January 2001; Revised 27 March 2001; Accepted 4 April 2001

**TABLE I** – PATIENT CHARACTERISTICS

| | Patients with locally advanced primary tumors | Patients with locally recurrent tumors |
|---|---|---|
| Menstrual status | | |
| Pre-menopause | 7 | 4 |
| Post-menopause | 6 | 8 |
| Performance status | | |
| 0 | 13 | 9 |
| 1 | 0 | 2 |
| 2 | 0 | 1 |
| Stage | | |
| II | 6 | 7[1] |
| III | 7 | 3 |
| Unknown | 0 | 2 |
| Histological type | | |
| Invasive ductal carcinoma | 11 | 11[1] |
| Invasive lobular carcinoma | 0 | 0 |
| Others | 2 | 0 |
| Unknown | 0 | 1 |
| Histological grade | | |
| I | 4 | 1[1] |
| II | 6 | 7 |
| III | 3 | 2 |
| Unknown | 0 | 2 |
| Estrogen receptor | | |
| Positive | 7 | 6 |
| Negative | 6 | 6 |
| Disease-free interval | | |
| Median (range) (months) | N.A.[2] | 26 (5–79) |

[1]Stage, histological type and grade of primary breast tumor.–[2]Not applicable.

**TABLE II** – THERAPY PRIOR TO DOC IN PATIENTS WITH LOCALLY RECURRENT TUMORS

| Therapy | Number of patients |
|---|---|
| Adjuvant therapy[1] | |
| None | 1 |
| Hormonotherapy | 0 |
| Chemotherapy | 5 |
| Hormono chemotherapy | 6 |
| Treatment after recurrence[2] | |
| Hormonotherapy | |
| None | 5 |
| One regimen | 4 |
| Two regimens | 3 |
| Chemotherapy | |
| None | 3 |
| One regimen | 6 |
| Two regimens | 3 |

[1]Hormonotherapy (tamoxifen), chemotherapy (CMF) and hormonochemotherapy (CMF + tamoxifen).–[2]Hormonotherapy (tamoxifen or medroxyprogesterone) and chemotherapy (CEF or 5′DFUR).–C, cyclophosphamide; M, methotrexate; F, 5-fluorouracil; E, epirubicin; 5′DFUR, 5′-deoxyfluorouridine.

*Primers, probes and real-time PCR*

Primers and probes for the *BRCA1* and *BRCA2* target genes were determined with the assistance of the computer program *Primer Express* (Perkin-Elmer Applied Biosystems, Foster City, CA) and selected so that they were located at different exons (exons 22 and 23 for *BRCA1* and exons 25 and 26 for *BRCA2*), to prevent amplification from contaminated genomic DNA. The sequences of probes for *BRCA1* and *BRCA2* were 5′-CATCATTCACCCTTGGCACAGGTGT-3′ and 5′-TGATCCCAAGTGGTCCACCCCAAC-3′, respectively. Both probes were labeled by FAM fluorescent spectrum as a reporter. Amplification primer pairs were 5′-ACAGCTGTGTGGTGCTTCTGTG-3′ and 5′-CATTGTCCTCTGTCCAGGCATC-3′ for *BRCA1* and 5′-CTTGCCCCTTTCGTCTATTTG-3′ and 5′-TACGGCCCTGAAGTACAGTCTT-3′ for *BRCA2*. PCRs were carried out using the ABI

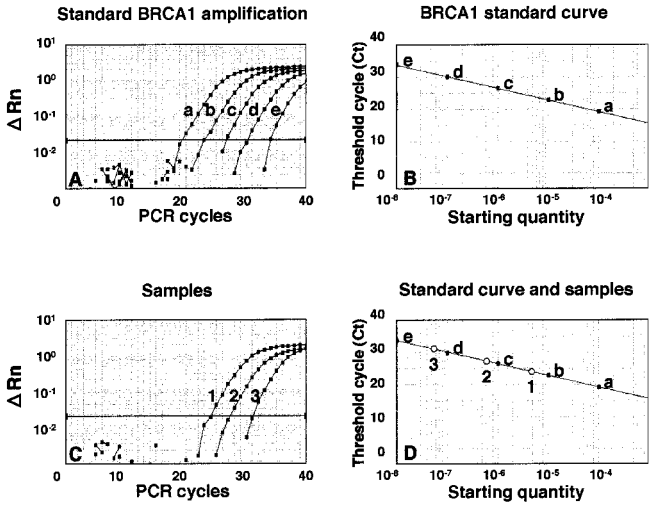


**FIGURE 1** – *BRCA1* standard curve by real-time RT-PCR. *(a)* Amplification plots. Samples containing 5 different dilutions (a, $10^{-4}$; b, $10^{-5}$; c, $10^{-6}$; d, $10^{-7}$; e, $10^{-8}$) of standard plasmids for *BRCA1* were subjected to real-time PCR. Cycle number is plotted *vs.* change in normalized reporter signal (ΔRn). For each reaction tube, the fluorescence signal of the reporter dye (dye for *BRCA1* and *BRCA2* was FAM, dye for β-glucuronidase was VIC) was divided by the fluorescence signal of the passive reference dye (TAMRA), to obtain a ratio defined as the normalized reporter signal (Rn). ΔRn represents the normalized reporter signal (Rn) minus the baseline signal established in the first 15 PCR cycles. ΔRn increases during PCR as the *BRCA1* PCR product copy number increases until the reaction reaches a plateau. $C_t$ represents the fractional cycle number at which a significant increase in Rn above the baseline signal (horizontal black line) can first be detected. Two replicates for each standard curve point sample (a–e) were performed, but the data for only 1 are shown. *(b)* Standard curve plotting log starting copy number *vs.* $C_t$. *(c)* Representative results of real-time PCR for *BRCA1* mRNA levels in 3 tumor tissues (samples 1–3). *(d)* Calculation of *BRCA1* mRNA levels (samples 1–3) according to the standard curve.

Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems) with a total volume of 50 μl reaction mixture containing 1 μl of cDNA template, 25 μl TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems), 0.1 μM probe and 0.3 μM of each primer. PCR conditions for *BRCA1* and *BRCA2* were as follows: after incubation at 50°C for 2 min and denaturing at 95°C for 10 min, 40 cycles at 95°C for 15 sec and 61°C for 1 min. To quantify gene transcripts precisely, we monitored the β-glucuronidase transcripts as the quantitative control and each sample was normalized on the basis of its β-glucuronidase transcript content. The primer probe mixture for β-glucuronidase was purchased from Perkin-Elmer Applied Biosystems and the method of PCR followed the manufacturer's protocol. Briefly, 50 μl reaction mixture containing 1 μl cDNA template, 25 μl TaqMan Universal PCR Master Mix and 2.5 μl primer probe mixture were amplified by the program as follows: after incubation at 50°C for 2 min and denaturing at 95°C for 10 min, 40 cycles at 95°C for 15 sec and at 60°C for 1 min.

Standard curves for *BRCA1*, *BRCA2* and β-glucuronidase mRNA were generated using serially diluted solutions ($10^{-4}$ to $10^{-8}$) of plasmid clones with either *BRCA1, BRCA2* or β-glucuronidase cDNA inserted as template (Fig. 1*a*). The parameter $C_t$ was designed as the fractional cycle number at which the fluorescence signal was induced, resulting from cleavage of the probe above the threshold level. The amount of target gene expression was calculated from the standard curve (Fig. 1*b*), and quantitative normalization of cDNA in each sample was performed using expression of the β-glucuronidase gene as an internal control. Finally, *BRCA1* and *BRCA2* mRNA levels were shown as ratios to β-glucuronidase mRNA levels. Real-time PCR assays were con-

ducted in duplicate for each sample, and the mean value was used for calculation of mRNA expression levels.

*Immunohistochemical assay*

Expression of P-gp, p53, erbB2, MIB1, BCL2 and ER-α was assessed by immunohistochemistry. Characteristics of antibodies are summarized in Table III. Sections (4 μm thick) from formalin-fixed, paraffin-embedded tissue blocks were incubated with each dilution of antibody. After incubation, specimens were processed using the avidin-biotin-peroxidase complex method, to detect the accumulation of each protein. Positive tumor cells were quantified by evaluating at least 1,000 cells and expressed as percentages. All samples were evaluated in a blinded procedure, without knowledge of the clinical outcome.

*Evaluation of chemotherapeutic response*

Chemotherapeutic response was clinically evaluated as follows: *(i)* complete response (CR), disappearance of all known disease; *(ii)* partial response (PR), ≥50% decrease in tumor size; *(iii)* no change (NC), <50% decrease or <25% increase in tumor size; *(iv)* progressive disease (PD), ≥25% increase in tumor size or appearance of new lesions. CR and PR were defined as responders and NC and PD, as non-responders.

*Statistical methods*

*BRCA1* and *BRCA2* mRNA expression and P-gp, p53, erbB2, MIB1, BCL2 and ER-α expression were compared between responders and non-responders by Student's *t*-test (Fig. 2). Tumors were dichotomized into groups with high or low *BRCA2* mRNA expression according to an arbitrary cut-off value (0.13), and the relationship between *BRCA2* mRNA expression and response to DOC was analyzed by the $\chi^2$ test. Statistical significance was set at $p < 0.05$.

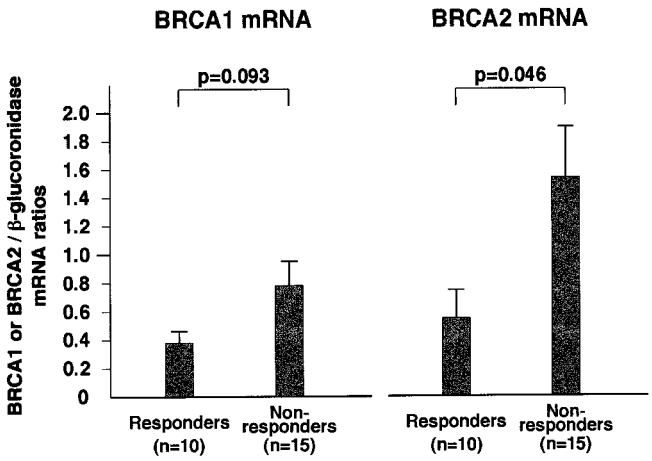


**FIGURE 2** – Comparison of *BRCA1* and *BRCA2* mRNA levels determined by real-time PCR between responders and non-responders to DOC treatment. Bars = SE.

## RESULTS

*Relationship between* BRCA1 *and* BRCA2 *mRNA expression and clinical response to DOC treatment*

We analyzed 13 patients with locally advanced breast tumors and 12 with locally recurrent tumors. Of these 25 patients, 10 showed a response (CR+PR) to DOC treatment with a response rate of 40%. *BRCA1* and *BRCA2* mRNA expression levels in the tumors were determined by real-time PCR. *BRCA1* mRNA levels (0.623 ± 0.114) were significantly ($p < 0.05$) lower than *BRCA2* mRNA levels (1.142 ± 0.246). *BRCA1* and *BRCA2* mRNA levels were compared between responders (CR+PR, n = 10) and non-responders (NC+PD, n = 15). *BRCA1* mRNA levels of responders showed a non-significant ($p = 0.09$) trend toward a decrease compared with those of non-responders, and *BRCA2* mRNA levels of responders were significantly ($p < 0.05$) lower than those of non-responders.

The relationship between *BRCA2* mRNA levels and response to DOC treatment is shown in Table IV. The response rate (40%) of tumors with high *BRCA2* mRNA levels was significantly ($p < 0.01$) lower than that (100%) of tumors with low *BRCA2* mRNA levels. Positive predictive value, negative predictive value and diagnostic accuracy of the *BRCA2* mRNA assay in the prediction of response to DOC were 100%, 75% and 80%, respectively.

*Comparison of expression of various biological markers determined by immunohistochemistry between responders and non-responders to DOC treatment*

The expression status of various biological markers (P-gp, p53, erbB2, MIB1, BCL2 and ER-α; Table V) was studied by immunohistochemistry, and the results (% of immunohistochemically positive cancer cells) were compared between responders and non-responders. No significant difference was found between responders and non-responders in the expression status of any of these 6 markers.

## DISCUSSION

We have shown that *BRCA2* mRNA levels are significantly lower in responders than non-responders to DOC treatment and suggest that *BRCA2* mRNA status can be used as a predictor of response to DOC treatment. Our observation that tumors with low *BRCA2* mRNA levels are more sensitive to DOC treatment appears to be consistent with the thesis that DOC exerts its antineoplastic effect more easily in tumors where the function of mitotic spindles is already retarded to some extent due to low expression of *BRCA2*. By setting an arbitrary cut-off value, we have also indicated that *BRCA2* mRNA levels can be used as a predictor of response to DOC treatment with a positive predictive value of 100%, a negative predictive value of 75% and a diagnostic accuracy of 80%. This possibility deserves further study, including a larger number of patients, since no clinically useful predictor of response to DOC treatment is available.

*BRCA1* mRNA levels, like *BRCA2*, were also lower in responders than non-responders, but the difference was statistically not significant ($p = 0.09$). *BRCA2* mRNA expression is both up-regulated and down-regulated in breast tumors, but *BRCA1* mRNA expression is down-regulated in almost all sporadic breast cancers compared with normal breast tissues.[14–16] Consistently, we have

**TABLE III** – ANTIBODY CHARACTERISTICS

| Antibody | Directed against | Vendor/donation | Mono-/polyclonal | Host species | Antibody class | Dilution |
|---|---|---|---|---|---|---|
| C219 | P-gp | CIS bio international (Yvette, France) | Monoclonal | Mouse | $IgG_{2b}$ | 1:50 |
| DO-7 | p53 | Dako (Glostrup, Denmark) | Monoclonal | Mouse | $IgG_{2b}$ | 1:100 |
| c-erbB2 | c-erbB2 | Nichirei (Tokyo, Japan) | Polyclonal | Rabbit | IgG | 1:100 |
| bcl-2 (124) | bcl-2 | Dako | Monoclonal | Mouse | $IgG_1$ | 1:100 |
| MIB-1 | Ki-67 | Immunotech (Marseille, France) | Monoclonal | Mouse | $IgG_1$ | 1:100 |
| ERα (H-184) | ERα | Santa Cruz Biotechnology (Santa Cruz, CA) | Polyclonal | Rabbit | IgG | 1:100 |

**TABLE IV** – RELATIONSHIP BETWEEN BRCA2 mRNA LEVELS AND RESPONSE TO DOC TREATMENT

| | Responders | Non-responders | *p* |
|---|---|---|---|
| BRCA2 mRNA[1] | | | |
| High | 5[2] | 15 | 0.0022 |
| Low | 5 | 0 | |

[1]BRCA2 mRNA levels were dichotomized into high and low groups according to an arbitrary cut-off value (0.13).–[2]Number of patients.

**TABLE V** – ASSOCIATION BETWEEN EXPRESSION OF BIOLOGICAL MARKERS AND RESPONSE TO DOC

| | Responders | Non-responders | *p* |
|---|---|---|---|
| P-gp | 8.2 ± 1.7[1] | 8.6 ± 1.6 | 0.887 |
| p53 | 15.8 ± 5.4 | 23.6 ± 6.9 | 0.436 |
| erbB2 | 12.5 ± 6.3 | 17.8 ± 6.6 | 0.597 |
| MIB1 | 32.2 ± 5.4 | 31.0 ± 3.9 | 0.859 |
| BCL2 | 24.5 ± 9.4 | 20.0 ± 6.1 | 0.679 |
| ERα | 10.6 ± 2.1 | 17.2 ± 3.9 | 0.223 |

[1]Percentage of immunohistochemically positive cancer cells (mean ± SE).

found that *BRCA1* mRNA levels are significantly lower than *BRCA2* mRNA levels. Thus, almost all breast tumors are considered to have already suffered from a significant loss of *BRCA1* function. It is speculated that a further small decrease in *BRCA1* mRNA expression does not affect the sensitivity of tumors to DOC treatment as seen for *BRCA2* mRNA expression.

Several biological markers have often been studied for their clinical usefulness as predictors of response to doxorubicin treatment, including multidrug-resistance genes (P-gp, *etc.*), tumor-suppressor genes (p53, *etc.*), oncogenes (erbB2, *etc.*), mitotic activity markers (MIB1, *etc.*), apoptosis-related genes (BCL2, *etc.*) and hormone receptors (ER, *etc.*).[21–24] Some of these markers have been suggested to be clinically useful in the prediction of response to doxorubicin treatment. However, the clinical significance of these markers as predictors for DOC treatment has rarely been studied in breast-cancer patients. *In vitro* studies have shown that resistance to DOC can be induced by P-gp, which is a product of multidrug-resistance gene 1 (*MDR1*), because DOC is pumped out by P-gp like doxorubicin.[25] The clinical significance of P-gp expression in the prediction of doxorubicin resistance has been suggested,[21,22] but in the present study, we could not find a significant difference in P-gp expression between responders and non-responders to DOC treatment, indicating that P-gp does not play an important role in the acquisition of DOC resistance. Other multidrug-resistance proteins (MRP1, *etc.*) might be involved in the efflux of DOC from cancer cells.

*p53* is a tumor-suppressor gene that plays an important role in the $G_1$–S checkpoint; it is one of the most frequently studied markers as a predictor of response to doxorubicin in breast cancer.[24] *p53* mutation (loss of function) inhibits doxorubicin-induced apoptosis, resulting in drug resistance. Similarly, *p53* mutation serves as one of the resistance mechanisms to DOC treatment experimentally.[26,27] However, we could not find any significant difference in *p53* immunostaining between responders and non-responders. We also dichotomized the tumors into $p53^+$ and $p53^-$ groups, according to the cut-off value of 10% since this value can differentiate such tumors with high accuracy into mutation positive and negative groups. Again, we could not find a significant difference between responders (56%) and non-responders (60%) ($p = 0.831$, $\chi^2$ test), suggesting that *p53* status is not clinically useful as a predictor of response to DOC treatment. Since we estimated *p53* mutation status by immunohistochemistry, *p53* mutations leading to protein truncation (nonsense or frameshift mutations) were overlooked, but the incidence of such mutations is generally not high enough to change our conclusion.

Other markers studied here included erbB2, MIB1, BCL2 and ER-α, which have been frequently studied as predictors of response to doxorubicin-containing chemotherapy in breast cancers;[23] however, their clinical significance is unclear. We studied the possibility that these markers can serve as predictors of response to DOC treatment but were not able to find a significant difference in expression of any of them between responders and non-responders, indicating that they are unlikely to serve as clinically useful predictors of response to DOC treatment.

As a resistance mechanism to paclitaxel, which is another taxane as active as DOC in the treatment of breast cancer, change of expression levels of β-tubulin subtypes in cell culture[28] and mutation of the β-tubulin gene in lung cancers[29] have been suggested. DOC and paclitaxel are thought to exert their anti-neoplastic activity through similar mechanisms, but the fact that DOC is effective in 18% to 33% of breast-cancer patients who are resistant to paclitaxel strongly indicates that the resistance mechanisms to these 2 drugs are not identical and the above-mentioned resistance mechanisms to paclitaxel need to be investigated for DOC.[30,31]

In conclusion, we suggest that *BRCA2* mRNA levels in tumor tissues might be useful in the prediction of response to DOC treatment in breast-cancer patients and that the other markers (P-gp, p53, erbB2, BCL2, MIB1, ER-α) frequently studied as predictors of response to doxorubicin treatment are not useful for DOC treatment. Our preliminary observation on *BRCA2* needs to be confirmed at the protein level in a larger number of patients after suitable antibodies become available.

## REFERENCES


1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66–71.
2. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994;265:2088–90.
3. Merajver SD, Frank TS, Xu J, Pham TM, Calzone KA, Bennett-Baker P, et al. Germline BRCA1 mutations and loss of the wild-type allele in tumors from families with early onset breast and ovarian cancer. Clin Cancer Res 1995;1:539–44.
4. Gudmundsson J, Johannesdottir G, Bergthorsson JT, Arason A, Ingvarsson S, Egilsson V, et al. Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13. Cancer Res 1995;55:4830–2.
5. Welsch PL, Owens KN, King MC. Insights into the functions BRCA1 and BRCA2. Trends Genet 2000;16:69–74.
6. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, et al. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 1997;88:265–75.
7. Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, et al. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 1998;2:317–28.
8. Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, et al. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cells 1999;3:389–95.
9. Miyoshi Y, Iwao K, Takahashi U, Egawa C, Noguchi S. Acceleration of chromosomal instability by loss of BRCA1 expression and p53 abnormality in sporadic breast cancers. Cancer Lett 2000;159:211–6.
10. Khoo US, Ozcelik H, Cheung AN, Chow LW, Ngan HY, Done SJ, et al. Somatic mutations in the BRCA1 gene in Chinese sporadic breast and ovarian cancer. Oncogene 1999;18:4643–6.
11. Weber BH, Brohm M, Stec I, Backe J, Caffier H. A somatic truncating mutation in BRCA2 in a sporadic breast tumor. Am J Hum Genet 1996;59:962–4.
12. Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, et al. BRCA2 mutations in primary breast and ovarian cancers. Nat Genet 1996;13:238–40.
13. Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. Mutation analysis in the BRCA2 gene in primary breast cancers. Nat Genet 1996;13:245–7.
14. Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. De-

creased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. Nat Genet 1995;9:444–50.
15. Ozcelik H, To MD, Couture J, Bull SB, Andrulis IL. Preferential allelic expression can lead to reduced expression of BRCA1 in sporadic breast cancers. Int J Cancer 1998;77:1–6.
16. Bieche I, Nogues C, Lidereau R. Overexpression of BRCA2 gene in sporadic breast tumours. Oncogene 1999;18:5232–8 (1999).
17. Rice JC, Massey-Brown KS, Futscher BW. Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells. Oncogene 1998;17: 1807–12.
18. Collins N, Wooster R, Stratton MR. Absence of methylation of CpG dinucleotides within the promoter of the breast cancer susceptibility gene BRCA2 in normal tissues and in breast and ovarian cancers. Br J Cancer 1997;76:1150–6.
19. Fulton B, Spencer CM. Docetaxel. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of metastatic breast cancer. Drugs 1996;51:1075–92.
20. Ringel I, Horwitz SB. Studies with RP56976 (Taxotere): a semisynthetic analogue of Taxol. J Natl Cancer Inst 1991;83:288–91.
21. Veneroni S, Zaffaroni N, Daidone MG, Benini E, Villa R, Silvestrini R. Expression of P-glycoprotein and in vitro or in vivo resistance to doxorubicin and cisplatin in breast and ovarian cancers. Eur J Cancer 1994;30A:1002–7.
22. Chung HC, Rha SY, Kim JH, Roh JK, Min JS, Lee KS, et al. P-glycoprotein: the intermediate end point of drug response to induction chemotherapy in locally advanced breast cancer. Breast Cancer Res Treat 1997;42:65–72.
23. Colleoni M, Orvieto E, Nole F, Orlando L, Minchella I, Viale G, et al. Prediction of response to primary chemotherapy for operable breast cancer. Eur J Cancer 1999;35:574–9.
24. Bottini A, Berruti A, Bersiga A, Brizzi MP, Brunelli A, Gorzegno G, et al. p53 but not bcl-2 immunostaining is predictive of poor clinical complete response to primary chemotherapy in breast cancer patients. Clin Cancer Res 2000;6:2751–8.
25. Shirakawa K, Takara K, Tanigawara Y, Aoyama N, Kasuga M, Komada F, et al. Interaction of docetaxel ("Taxotere") with human P-glycoprotein. Jpn J Cancer Res 1999;90:1380–6.
26. Horio Y, Hasegawa Y, Sekido Y, Takahashi M, Roth JA, Shimokata K. Synergistic effects of adenovirus expressing wild-type p53 on chemosensitivity of non-small cell lung cancer cells. Cancer Gene Ther 2000;7:537–44.
27. Inoue A, Narumi K, Matsubara N, Sugawara S, Saijo Y, Satoh K, et al. Administration of wild-type p53 adenoviral vector synergistically enhances the cytotoxicity of anti-cancer drugs in human lung cancer cells irrespective of the status of p53 gene. Cancer Lett 2000;157: 105–12.
28. Kavallaris M, Kuo DYS, Burkhart CA, Regl DL, Norris MD, Haber M, et al. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes. J Clin Invest 1997;100:1282–93.
29. Monzo M, Rosell R, Sanchez JJ, Lee JS, O'Brate A, Gonzalez-Larriba JL, et al. Paclitaxel resistance in non-small-cell lung cancer associated with beta-tubulin gene mutations. J Clin Oncol 1999;17:1786–93.
30. Valero V, Jones SE, von Hoff DD, Booser DJ, Mennel RG, Ravdin PM, et al. A phase II study of docetaxel in patients with paclitaxel-resistant metastatic breast cancer. J Clin Oncol 1998;16:3362–8.
31. Lin YC, Chang HK, Wang CH, Chen JS, Liaw CC. Single-agent docetaxel in metastatic breast cancer patients pre-treated with anthracyclines and paclitaxel: partial cross-resistance between paclitaxel and docetaxel. Anticancer Drugs 2000;11:617–21.