Expression of Multidrug Resistance-related Transporters in Human Breast Carcinoma

Atsuko Kanzaki,1 Masakazu Toi,2 Kentaro Nakayama,1 Hiroko Bando,2 Masato Mutoh,3 Takafumi Uchida,1 Manabu Fukumoto1 and Yuji Takebayashi1,4

1Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, 2Department of Surgery, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677 and 3Pharmaceutical Research Laboratories, Toray Industries, Inc., 1111 Tebiro, Kamakura, Kanagawa 248-8555

The expression levels of mRNA for multidrug resistance 1 (MDR1), multidrug resistance protein 1 (MRP1), lung resistance-related protein (LRP) and breast cancer resistance protein (BCRP), which confer multidrug resistance in vitro, were examined in 43 untreated breast carcinoma patients, of whom 38 subsequently received doxorubicin-based chemotherapy after surgery, in order to elucidate the roles of these genes in drug resistance in vivo. The mRNA levels were determined using a semi-quantitative reverse-transcription polymerase chain reaction method in breast carcinoma tissues including at least 80% carcinoma cells. The expression level of BCRP gene was low and did not vary markedly in comparison with that of MDR1, MRP1 or LRP gene. The expressions of MDR1 and MRP1 genes were correlated with each other, but the expression of BCRP or LRP gene did not correlate with that of other genes. These four gene expressions were independent of age, TNM categories and the status of progesterone or estrogen receptor. The expression levels of these four genes were not related to the relapse or prognosis of the 38 patients treated with doxorubicin-based chemotherapy. P-glycoprotein (P-gp)/MDR1, MRP1 and LRP may play more important roles than BCRP in chemotherapy of human breast carcinoma.

Key words: MDR — BCRP — Breast carcinoma

Intrinsic or acquired drug resistance is a major therapeutic problem for cancer chemotherapy, limiting the efficacy of chemotherapy.1) Knowledge of the mechanisms of drug resistance may lead to new treatment strategies for overcoming drug resistance.

MDR is an important mechanism of drug resistance in tumor cell lines, and involvement of MDR1, MRP1 and LRP genes has been identified.2)–4) MDR1 and MRP1 are members of the ABC transporter gene family,5)–6) expressed in both human solid tumors and hematological malignancies.7, 8) The 110-kd LRP, the major vault protein, is frequently overexpressed in multidrug-resistant cells, and has an important role(s) in transportation of drugs from nucleus to cytoplasm.4) Further, Akiyama and co-workers demonstrated that ribozyme to LRP gene resulted in doxorubicin, VP-16 and paclitaxel resistance in SW620 cells induced to differentiate by treatment with sodium butyrate.9) Recently, BCRP (MXR/ABCP) gene, a member of the ABC transporter family, has been described in breast, colon, gastric and fibrosarcoma cell lines.10–12) Overexpression of BCRP was induced by exposure of the cells to mitoxantrone or doxorubicin/verapamil and resulted in a different resistance profile from the cases of MDR1 or MRP1 gene overexpression.13, 14) Topoisomerase I inhibitors such as topotecan and camptothecin are substrates for BCRP.13–15)

Several reports have appeared on the expression of P-gp/MDR1, MRP1 and LRP in human breast carcinoma using immunohistochemistry or molecular biological methods (Table I). However, their clinical significance in human breast carcinoma is still uncertain. Further, the expression of BCRP in human samples has been analyzed only in hematological malignancy.16) In this study, we analyzed the expression levels of BCRP, MDR1, MRP1 and LRP genes in patients with untreated breast carcinoma.

MATERIALS AND METHODS

Patients and samples Surgical specimens from 43 patients with untreated breast carcinoma were available for this study. The patients underwent surgery in Tokyo Metropolitan Hospital between 1983 through 1999. All the samples were stored at −80°C and embedded in O.C.T. compound (Sakura Finetechnical Co., Ltd., Tokyo) immediately before use. The data on clinicopathologic variables such as age, TNM categories and the status of estrogen/
progesterone receptor are shown in Table I. The relapse and prognosis of the patients was investigated on June 30, 2000. Informed consent was obtained from each patient, and Tokyo Metropolitan Komagome Hospital committee approved this project prior to the study. The sections including at least 80% carcinoma cells were used for total RNA preparation.

RT-PCR Total RNA of human breast carcinoma was prepared by using Trizol (Gibco Life Tech, Gatesberg, MD). cDNA was synthesized with 3 µg of total RNA and random hexadeoxynucleotide primer (Gibco Life Tech) in 20 µl of a solution containing reverse transcriptase. cDNA was diluted 1:4 with water and stored at −20°C until use. PCR was performed with cDNA derived from 30 ng of RNA. PCR reactions were carried out in a total volume of 25 µl containing cDNA, dGTP, dATP, dTTP and [α-32P]dCTP at a concentration of 200 µM, 4 µM of each primer and 0.25 unit of ExTaq polymerase (TaKaRa Shuzo, Otsu, Shiga). The PCR condition consisted of 10 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C, followed by 72°C for 10 min. The PCR primer sequences of MDR1, MRP1, LRP, BCRP and GAPDH which was used as an internal control were as follows: MDR1 sense primer 5'-ATCAAGACCCGCTGTCATTGG-3' and antisense primer 5'-TCTCGTTCTCAGTGTCATCC-3' corresponding to 180-bp (residues 1379 to 1559); MRP1 sense primer 5'-CCCATACACAGAGTGTCATCCG-3' and antisense primer 5'-AACATTCAAGGCTTTCAGTA-3' corresponding to 275-bp (residues 1559 to 1834); LRP sense primer 5'-ATCATCCATCCAGGATCC-3' and antisense primer 5'-AGAGGGTTCCTCCGTGTCACAG-3' corresponding to 170-bp (residues 1834 to 2003); BCRP sense primer 5'-ATCATCCATCCAGGATCC-3' and antisense primer 5'-AGAGGGTTCCTCCGTGTCACAG-3' corresponding to 170-bp (residues 1834 to 2003).

PCR and quantitative analysis of PCR products In order to evaluate the amplified PCR products semi-quantitatively, the optimal conditions for the detection of MDR1, MRP1, LRP, BCRP and GAPDH genes were determined using cDNA derived from placenta. At 40 cycles of PCR, the relative yields of PCR products were similar, indicating that this number of cycles corresponded to the plateau. At 25 cycles or less, expression of each gene could not be clearly distinguished (data not shown). Thus we used 35 PCR cycles for the detection of each gene. The amplified cDNA fragment was electrophoresed on 6% polyacryl-
amide gel (Fig. 1). The quantitative analysis of the amplified PCR products was performed using a BAS 2000 imaging plate (Fuji, Kanagawa).

**Statistical analysis** Association of continuous variables was evaluated using the Mann-Whitney U test. The relationship between MDR1, MRP1, LRP or BCRP gene expressions and potential explanatory variables, including age, pT category, pN category, pM category and hormonal receptor status was determined by use of the χ² test. The statistical analyses were performed using Statview J 5.0 software (Abacus, CA). Two-sided P values were calculated and were considered significant when less than 0.05.

**RESULTS**

Expression and clinical significance of BCRP, MDR1, MRP1 or LRP genes in human breast carcinoma Fortyt-three primary breast carcinoma tissues were used for the detection of BCRP, MDR1, MRP1 or LRP genes. 43 patients with breast carcinoma, determined by RT-PCR, as described in “Materials and Methods.” Lanes C: RNA of placenta and HCT15 cells was used as positive controls for the detection of MDR1, MRP1, BCRP and LRP genes. Lanes W: PCR was performed without each cDNA. Data were expressed relative to the expression of GAPDH gene in each breast carcinoma.

| Case No. | 9 15 21 23 24 C W |
|----------|-------------------|
| MDR1     |                  |
| MRP1     |                  |
| LRP      |                  |
| BCRP     |                  |
| GAPDH    |                  |

**Table II. Relationship of MDR1, MRP, LRP and BCRP Gene Expression Levels and Clinicopathological Variables in Patients with Breast Carcinoma**

| Variables     | All patients | MDR1 | MRP | LRP | BCRP |
|---------------|--------------|------|-----|-----|------|
| Age (yr)      |              |      |     |     |      |
| Median        |              | 52   | 51  | 52  | 50   |
| Range         | (36–80)      | (31–80) | (31–80) | (30–65) | (31–80) | (30–65) |
| pT category   |              |      |     |     |      |
| T1            |              | 2    | 1   | 2   | 1    |
| T2            |              | 20   | 9   | 9   | 9    |
| T3            |              | 11   | 6   | 4   | 5    |
| T4            |              | 10   | 5   | 6   | 5    |
| pN category   |              |      |     |     |      |
| N0            |              | 11   | 6   | 5   | 45   |
| N1            |              | 26   | 13  | 12  | 46   |
| N2            |              | 2    | 2   | 2   | 100  |
| N3            |              | 2    | 2   | 1   | 100  |
| N4            |              | 2    | 2   | 1   | 100  |
| pM category   |              |      |     |     |      |
| M0            |              | 33   | 18  | 17  | 52   |
| M1            |              | 7    | 3   | 3   | 43   |
| M2            |              | 7    | 3   | 3   | 43   |
| M3            |              | 7    | 3   | 3   | 43   |
| ERa           |              |      |     |     |      |
| Positive      |              | 25   | 11  | 9   | 36   |
| Negative      |              | 17   | 9   | 6   | 65   |
| PRa           |              |      |     |     |      |
| Positive      |              | 26   | 10  | 11  | 42   |
| Negative      |              | 15   | 9   | 8   | 53   |

a) Mann-Whitney U test or χ² test was used.
b) NS, not significant.
c) Estrogen receptor.
d) Progesterone receptor.
Table II summarizes the patients’ characteristics at the time when the samples were obtained at surgery.

**MDR1, MRP1, LRP and BCRP gene expression levels** are shown in Fig. 2. Although **MDR1**, **MRP1**, **LRP** or **BCRP** gene expression varied more than 1000-fold overall, one very high expression case was found for each of **LRP** and **BCRP**: Excluding this, **BCRP** gene expression level did not differ much in comparison with **MDR1**, **MRP1** or **LRP** gene expression levels.

Based on the median value of the mRNA expression of each gene, we examined the relationship between clinicopathologic variables and each gene expression. The expression levels were independent of age, TNM category and status of estrogen/progesterone receptors (Table II), and every other category examined.

Concerning the effects of the expression levels of these genes on doxorubicin-based chemotherapy in human breast carcinoma, we analyzed the relationship between relapse and the expression levels of **MDR1**, **MRP1**, **LRP** and **BCRP** among 38 cases treated with doxorubicin after surgery. The proportions of the cases with relapse were relatively similar in relation to **MDR1** (first quartile, recurrence/cases, 4/9; second, 4/9; third, 6/9 and fourth, 4/11), **MRP1** (4/9, 5/9, 4/9, 4/11), **LRP** (5/9, 5/9, 4/9, 4/11) and **BCRP** gene expression levels (5/9, 4/9, 6/9, 3/11) (Fig. 2). Box-plot analysis also revealed no difference of expression levels between the cases with relapse and those without (Fig. 3). The expression levels of these genes did not appear to have any marked effect in patients with breast carcinoma (data not shown).

**BCRP gene expression is independent of MDR1, MRP1 or LRP** To observe the expression pattern of **MDR1**, **MRP1**, **LRP** and **BCRP** genes in human breast carcinoma, the expression levels were plotted in a graph (Fig. 4). A moderate correlation was observed between **MDR1** gene expression and **MRP1** ($R=0.515$). **BCRP** or **LRP** gene expression is independent of **MDR1**, **MRP1** or **LRP**. Fig. 3. Association of **MDR1**, **MRP1**, **LRP** or **BCRP** gene expression with relapse in 38 patients with breast carcinoma. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median. $P$ values were assessed by Student’s $t$ test.  recurrence, negative;  recurrence, positive.

**Fig. 2.** Expression levels are arranged in order of magnitude for **MDR1** (A), **MRP1** (B), **LRP** (C) and **BCRP** gene (D). The hatched bar and * indicate patients with relapse after surgery.
expression showed no association with any other gene (BCRP vs. MDR1, \( R = 0.012 \); vs. MRP1, \( R = 0.023 \); vs. LRP, \( R = 0.0256 \)).

**DISCUSSION**

Several mechanisms are thought to be involved in breast cancer resistance to chemotherapy, including overexpression of the membrane-associated ATP-dependent efflux pump, P-gp encoded by *MDR1*, *MRP1* and *BCRP*, increased level of thymidylate synthetase, altered expression of topoisomerase II, and enhanced detoxification by the glutathione-linked enzyme system.\(^{1, 17, 18}\) *MDR1/P-gp*-associated MDR is the best characterized and best understood at the molecular level.\(^{17, 18}\) However, the contribution of these genes to chemotherapeutic failure in breast carcinoma remains to be proven.\(^{18}\) Recently, a novel ATP-dependent transporter, BCRP has been identified.\(^{10–12}\) This transporter was also involved in resistance to mitoxantrone, doxorubicin, topotecan and camptothecin.\(^{13, 14}\) Therefore, we investigated *BCRP* gene expression level in human breast carcinoma tissues, together with that of *MDR1*, *MRP1* and *LRP* gene, in relation to the clinical data of the patients to clarify whether these genes are involved in clinical drug resistance.

The present study revealed low expression of *BCRP* and small differences of *BCRP* gene expression from tumor to tumor, compared with *MDR1*, *MRP1* or *LRP*. Recently, Scheper and co-workers demonstrated BCRP expression on cytoplasm membrane in several cell lines by immunocytochemical analysis with the antibody BXP-34 against BCRP. In addition, no expression of BCRP was observed in 16 hematological malignancies and 41 human solid tumors, including 17 breast carcinomas.\(^{19}\) BCRP may not play an important role in progression of breast carcinoma, because its gene expression levels were not also associated with clinicopathologic variables in our study. Similar results have been reported for P-gp/*MDR1*, *MRP1* and *LRP*.\(^{18, 20–23}\) By contrast, P-gp and MRP1 expression detected by immunohistochemistry was reported to be associated with tumor progression, histopathologic subtype and age in human breast carcinoma.\(^{24–26}\)

Fojo and co-workers found relationships between *MDR1*/*MRP1* gene expression level and drug sensitivity using 60 NCI drug-screening cell lines.\(^{27, 28}\) The association of doxorubicin sensitivity and *MDR1*/*MRP1* gene expression was weak in their reports. Furthermore, *BCRP*, *MDR1*, *MRP1* or *LRP* gene expression did not influence the relapse and prognosis of the patients with breast carcinoma treated with doxorubicin in our present study.

---

**Fig. 4.** Correlation among *MDR*, *MRP1*, *LRP* or *BCRP* gene expression in breast carcinomas. Expression of *MDR*, *MRP1*, *LRP* or *BCRP* gene in 43 patients with breast carcinoma, determined by RT-PCR, as described in "Materials and Methods." Each gene expression level is reported relative to GAPDH gene. A. *MDR1* vs. *MRP1* gene expression. B. *MDR1* vs. *LRP* gene expression. C. *MDR1* vs. *BCRP* gene expression. D. *MRP1* vs. *LRP* gene expression. E. *MRP1* vs. *BCRP* gene expression. F. *BCRP* vs. *LRP* gene expression.
In previous reports, P-gp expression detected by immuno- 
histochemistry could predict doxorubicin response in 
human breast carcinoma, but MRP1 or LRP expression 
could not. Therefore, immunohistochemical analysis 
appears to be a better tool for the detection of these pro-

teins than RT-PCR. However, the condition of each 
section may not always be the same, so in this 
respect, RT-PCR could be a better tool for analysis of 
MDR1, MRP1, LRP or BCRP gene. First we examined the 
ratio of carcinoma cells on each section on the H.E. staining 
image and we used the sections including at least 80% 
carcinoma cells. We used GAPDH as an internal control to 
choose good quality sections for RT-PCR. Five cases were 

excluded because of the low ratio of RNA (<1.4) or fail-
ure to detect GAPDH by RT-PCR. However, there still 
remain problems of methodology in the detection of P-gp/ 
MDR1, MRP1, LRP and BCRP in human samples.

The co-expression of MDR1 and MRP1 was observed in 
breast carcinoma tissues in this study. In contrast, no associ-
ation between the expression of BCRP or LRP and any 
other gene was observed. Ross and co-workers reported 
that high expression of BCRP did not strongly correlate 
with high MDR1 expression. Thus, BCRP may not play an 
important role in human breast carcinoma. However, the 
significance of BCRP as well as P-gp/MDR1, MRP1 
and LRP in patients with carcinoma expressing these pro-
teins is not still clear. To establish the real significance of 
these transporters, it may be necessary to observe the 
clinical effects of specific inhibitors of each transporter.

(Received December 5, 2000/Revised February 1, 2001/ 
Accepted February 3, 2001)

REFERENCES

1) Gottesman, M. M. and Pastan, I. Biochemistry of multi-
drug resistance mediated by the multidrug transporter. 
Annu. Rev. Biochem., 62, 385–427 (1993).

2) Riordan, J. R., Deuchars, K., Kartner, N., Alon, N., Trent, J. 
and Ling, V. Amplification of P-glycoprotein genes in mul-
tidrug-resistant mammalian cell lines. Nature, 316, 817– 
819 (1985).

3) Cole, S. P. and Deeley, R. G. Multidrug resistance-associated 
gene: sequence correction [letter; comment]. Science, 260, 879 (1993).

4) Scheffer, G. L., Wijngaard, P. L., Flens, M. J., Izquierdo, 
M. A., Slovak, M. L., Pinedo, H. M., Meijer, C. J., Clevers, 
H. C. and Schepers, R. J. The drug resistance-related protein 
LRP is the human major vault protein. Nat. Med., 1, 578– 
582 (1995).

5) Ueda, K., Cardarelli, C., Gottesman, M. M. and Pastan, I. 
Expression of a full-length cDNA for the human “MDR1” 
gene confers resistance to colchicine, doxorubicin, and vin-
blastine. Proc. Natl. Acad. Sci. USA, 84, 3004–3008 
(1987).

6) Cole, S. P., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. 
E., Wilson, G. M. and Deeley, R. G. Pharmacological charac-
terization of multidrug resistant MRP-transfected human 
tumor cells. Cancer Res., 54, 5902–5910 (1994).

7) Goldstein, L. J., Galski, H., Fojo, A., Willingham, M., Lai, S. 
L., Gazdar, A., Pirker, R., Green, A., Crist, W., Brodeur, G. 
M., Lieber, M., Cossman, J., Gottesman, M. M. and Pastan, I. 
Expression of a multidrug resistance gene in human cancers. 
J. Natl. Cancer Inst., 81, 116–124 (1989).

8) Nooter, K., Bosman, F. T., Burger, H., van Wingerden, K. 
E., Flens, M. J., Scheper, R. J., Oostrum, R. G., Boersma, 
A. W., van der Gaast, A. and Stoter, G. Expression of the 
multidrug resistance-associated protein (MRP) gene in pri-
mary non-small-cell lung cancer. Ann. Oncol., 7, 75–81 
(1996).

9) Kitazono, M., Sumizawa, T., Takebayashi, Y., Chen, Z. S., 
Furukawa, T., Nagayama, S., Tani, A., Takao, S., Aikou, T. 
and Akiyama, S. Multidrug resistance and the lung resis-
tance-related protein in human colon carcinoma SW-620 
cells [see comments]. J. Natl. Cancer Inst., 91, 1647–1653 
(1999).

10) Allikmets, R., Schriml, L. M., Hutchinson, A., Romano-
Spica, V. and Dean, M. A human placenta-specific ATP-
binding cassette gene (ABCP) on chromosome 4q22 that is 
involved in multidrug resistance. Cancer Res., 58, 5337– 
5339 (1998).

11) Miyake, K., Mickley, L., Litman, T., Zhan, Z., Robey, R., 
Cristensen, B., Brangi, M., Greenberger, L., Dean, M., 
Fojo, T. and Bates, S. E. Molecular cloning of cDNAs 
which are highly overexpressed in mitoxantrone-resistant 
cells: demonstration of homology to ABC transport genes. 
Cancer Res., 59, 8–13 (1999).

12) Doyle, L. A., Yang, W., Abruzzo, L. V., Krogmann, T., 
Gao, Y., Rishi, A. K. and Ross, D. D. A multidrug resis-
tance transporter from human MCF-7 breast cancer cells. 
Proc. Natl. Acad. Sci. USA, 95, 15665–15670 (1998).

13) Allen, J. D., Brinkhuis, R. F., Wijnholds, J. and Schinkel, 
A. H. The mouse Bcrp1/Mxr/Abcp gene: amplification 
and overexpression in cell lines selected for resistance to 
topotecan, mitoxantrone, or doxorubicin. Cancer Res., 59, 
4237–4241 (1999).

14) Volk, E. L., Rohde, K., Rhein, M., McGuire, J. J., Doyle, L. 
A., Ross, D. D. and Schneider, E. Methotrexate cross-resis-
tance in a mitoxantrone-selected multidrug-resistant MCF7 
breast cancer cell line is attributable to enhanced energy-
dependent drug efflux [In Process Citation]. Cancer Res., 
59, 4237–4241 (1999).
15) Brangl, M., Litman, T., Ciotti, M., Nishiyama, K., Kohlhagen, G., Takimoto, C., Robey, R., Pommier, Y., Fojo, T. and Bates, S. E. Camptothein resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res., 59, 5938–5946 (1999).

16) Ross, D. D., Karp, J. E., Chen, T. T. and Doyle, L. A. Expression of breast cancer resistance protein in blast cells from patients with acute leukemia. Blood, 96, 365–368 (2000).

17) Fisher, G. A. and Sikic, B. I. Drug resistance in clinical oncology and hematology. Introduction. Hematol. Oncol. Clin. North Am., 9, xi–xii (1995).

18) Wallner, J., Depisch, D., Hopfner, M., Haider, K., Spona, J., Ludwig, H. and Pirker, R. MDR1 gene expression and prognostic factors in primary breast carcinomas. Eur. J. Cancer, 27, 1352–1355 (1991).

19) Scheffer, G. L., Maliepaard, M., Pijnengen, A. C., van Gastelen, M. A., de Jong, M. C., Schroeijers, A. B., van der Kolk, D. M., Allen, J. D., Ross, D. D., van der Valk, P., Dalton, W. S., Schellens, J. H. and Scheper, R. J. Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res., 60, 2589–2593 (2000).

20) Lacave, R., Coulet, F., Ricci, S., Touboul, E., Flahault, A., Rateau, J. G., Cesari, D., Lefranc, J. P. and Bernaudin, J. F. Comparative evaluation by semi-quantitative reverse transcriptase polymerase chain reaction of MDR1, MRP and GSTP gene expression in breast carcinomas. Br. J. Cancer, 77, 694–702 (1998).

21) Hegewisch-Becker, S., Staib, F., Loning, T., Pichlmayr, U., Kroger, N., Reynmann, A. and Hossfeld, D. K. No evidence of significant activity of the multidrug resistance gene product in human primary breast cancer. Ann. Oncol., 9, 85–93 (1998).

22) Yang, X., Uziely, B., Groschen, S., Lukas, J., Israel, V., Russell, C., Dunnington, G., Formenti, S., Muggia, F. and Press, M. F. MDR1 gene expression in primary and advanced breast cancer. Lab. Invest., 79, 271–280 (1999).

23) Pohl, G., Filipits, M., Suchomel, R. W., Stranzl, T., Depisch, D. and Pirker, R. Expression of the lung resistance protein (LRP) in primary breast cancer. Anticancer Res., 19, 5051–5055 (1999).

24) Charpin, C., Vielli, P., Duffaud, F., Devictor, B., Andrac, L., Lavaut, M. N., Allasia, C., Horschowski, N. and Piana, L. Quantitative immunocytochemical assays of P-glycoprotein in breast carcinomas: correlation to messenger RNA expression and to immunohistochemical prognostic indicators. J. Natl. Cancer Inst., 86, 1539–1545 (1994).

25) Filipits, M., Suchomel, R. W., Dekan, G., Haider, K., Väldimarsson, G., Depisch, D. and Pirker, R. MRP and MDR1 gene expression in primary breast carcinomas. Clin. Cancer Res., 2, 1231–1237 (1996).

26) Nooter, K., Brutel de la Riviere, G., Look, M. P., van Wingerden, K. E., Henzen-Logmans, S. C., Schepers, R. J., Flens, M. J., Klijn, J. G., Stoter, G. and Foekens, J. A. The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. Br. J. Cancer, 76, 486–493 (1997).

27) Alvarez, M., Paull, K., Monks, A., Hose, C., Lee, J. S., Weinstein, J., Grever, M., Bates, S. and Fojo, T. Generation of a drug resistance profile by quantitation of mdr-1/P-glycoprotein in the cell lines of the National Cancer Institute Anticancer Drug Screen. J. Clin. Invest., 95, 2205–2214 (1995).

28) Alvarez, M., Robey, R., Sandor, V., Nishiyama, K., Matsumoto, Y., Paull, K., Bates, S. and Fojo, T. Using the national cancer institute anticancer drug screen to assess the effect of MRP expression on drug sensitivity profiles. Mol. Pharmacol., 54, 802–814 (1998).

29) Mechetner, E., Kyshtoobayeva, A., Zonis, S., Kim, H., Stroup, R., Garcia, R., Parker, R. J. and Fruehauf, J. P. Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin. Clin. Cancer Res., 4, 389–398 (1998).

30) Chevillard, S., Pouillart, P., Beldjord, C., Asselain, B., Beuzeboc, P., Magdelenaat, H. and Vielh, P. Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. Cancer, 77, 292–300 (1996).

31) Linn, S. C., Hoekman, A. H., Hoekman, K., van der Valk, P., Pinedo, H. M. and Giaccone, G. p53 and P-glycoprotein are often co-expressed and are associated with poor prognosis in breast cancer. Br. J. Cancer, 74, 63–68 (1996).

32) Beck, W. T., Grogan, T. M., Willman, C. L., Carbone, P. A., Demetri, G. D., Houghton, P. J., Sivridis, D. K., Lehrner, M., Leith, C. P., Paitel, A., Pavelic, Z. P. and Weinstein, R. Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. Cancer Res., 56, 3010–3020 (1996).

33) Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Giaccone, G., Broxterman, H. J., Meijer, C. J., van der Valk, P. and Schepers, R. J. Broad distribution of the multidrug resistance-associated vault lung resistance protein in normal human tissues and tumors. Am. J. Pathol., 148, 877–887 (1996).

34) Linn, S. C., Pinedo, H. M., van Ark-Otte, J., van der Valk, P., Hoekman, K., Hoekman, A. H., Vermorken, J. B. and Giaccone, G. Expression of drug resistance proteins in breast cancer, in relation to chemotherapy. Int. J. Cancer, 71, 787–795 (1997).