

ORIGINAL ARTICLE

The use of an in vitro adenosine triphosphate-based chemotherapy response assay to predict chemotherapeutic response in breast cancer

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Summary

The adenosine triphosphate-based chemotherapy response assay (ATP-CRA) has the advantages of standardization, evaluability, reproducibility, and accuracy, and can be performed on relatively small numbers of tumor cells. A total of 43 patients were enrolled in the present study, and chemosensitivity tests were successfully performed in 40 (93.0%) of these patients. Twenty of the 40 received neoadjuvant chemotherapy or chemotherapy for metastatic breast cancer. The chemotherapy regimens used were doxorubicin plus docetaxel ($n = 9$, 45.0%) or doxorubicin plus paclitaxel ($n = 11$, 55.0%). Mean cell death rate, as determined by ATP-CRA, was lower in non-responders than in responders to therapy ($P = 0.012$). Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for ATP-CRA were 78.6%, 100%, 100%, 66.7%, and 85.0%, respectively. Diagnostic accuracy achieved by immunohistochemistry using estrogen receptor or progesterone receptor was lower than that achieved using ATP-CRA. Expression of p53, erb-B2, Ki67, Bcl-2, Bcl-xL, and annexin I was not significantly associated with response to chemotherapy. Our results show that ATP-CRA has high specificity and positive predictive value for predicting response to chemotherapy.

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Introduction

In 2000, breast cancer became the most common cancer in women in the Republic of Korea, and its incidence is gradually increasing globally.^{1–3} Although many successful developments have been made over the past few decades in the field of breast cancer treatment, it remains a highly dangerous disease.

Systemic chemotherapy plays a key role in the treatment of breast cancer patients. However, a substantial number

of patients suffer from considerable side effects and do not respond to treatment.

Moreover, it is generally accepted that a strong correlation exists between pathologic complete response (CR) after neoadjuvant chemotherapy and overall and disease-free survival.^{4–7} Thus, it is reasonable to expect that increases in response rates to chemotherapy are likely to improve overall and disease-free survival. Accordingly, several studies have been undertaken to develop methods capable of predicting tumor response to chemotherapy. The two main strategies used involve the identification of meaningful biological predictors and in vitro chemosensitivity testing. In terms of the former method, although

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some biological markers have shown potential, it is unclear at present which biological markers helpfully predict response to chemotherapy in individual patients.⁸ On the other hand, some in vitro chemosensitivity tests are already available, e.g., the human tumor clonogenic assay (HTCA), the thymidine incorporation assay (TIA), the succinic dehydrogenase inhibition test (SDI test), 3-[4,5-dimethyl-ethiazol-2-yl]-2, the 5-diphenyltetrazolium bromide (MTT) assay, the differential staining cytotoxicity (DiSC) assay, the three-dimensional agarose-based extreme drug-resistance assay (EDRA), and the histoculture drug-response assay (HDRA).^{9–14} However, these tests and assays are not used in clinical practice in the neoadjuvant setting nor do they determine the likelihood of chemotherapeutic success in a metastatic setting. This failure is probably due to the low success rates of these modalities in primary cell cultures, their requirements of large numbers of tumor cells, and the excessive amount of time required.

Intracellular ATP is the basic energy source for all living cells, and rapidly disappears when cells lose viability. Thus, ATP assays can analyze the cytotoxic effects of anticancer drugs.¹⁵ The adenosine triphosphate-based chemotherapy response assay (ATP-CRA) was found to have a high success rate in primary culture and to require only a small number of cells. In addition, it eliminates problems caused by fibroblast contamination and can be performed relatively quickly.^{16–18} Moreover, recent studies have found that ATP-CRA results predict chemosensitivity in patients with ovarian cancer or gastrointestinal cancer.^{18–20}

The purpose of this study was to investigate the clinical applicability and accuracy of ATP-CRA as a predictor of chemotherapeutic response in patients with breast cancer. In addition, the authors evaluated the expression of several biological markers that have previously been suggested to predict chemotherapeutic response in breast cancer.

Materials and methods

Patients

Forty-three patients newly diagnosed with breast cancer at Mokdong Hospital, Ewha Womans University or the Gil Medical Center, Gachon Medical School between December 2004 and February 2005 were enrolled in this study. All patients were diagnosed as having invasive ductal carcinoma by a pathologist. When a tumor contained more than 80% of a ductal carcinoma in situ component, the patient was excluded. Informed consent was obtained from all study participants.

Bilateral mammography, bilateral breast ultrasonography, bone scan, abdominal ultrasonography, simple chest X-ray, serum CA15-3, serum CEA, liver function test, and renal function test were conducted in all participants for initial screening. Breast lesions were measured by ultrasonography or MRI. Two or three specimens were taken per patient by tru-cut biopsy for ATP-CRA testing.

Chemotherapy regimens were selected by the doctors responsible without any knowledge of the ATP-CRA results. Response to chemotherapy was evaluated after a minimum of two chemotherapy cycles. Breast ultrasound and/or MRI were repeated at the end of the second or third chemotherapy cycle. Tumor size was calculated using the two-dimensional method, i.e., tumor size = maximal length × maximal width. These calculations were performed before and after chemotherapy to determine response rates. Within 21 days of completing chemotherapy, patients with operable breast cancer underwent surgery and postoperative treatment according to local policy. All surgically obtained specimens were evaluated by a pathologist and staged according to the TNM system. Pathologic results were used to confirm a radiologic finding of CR. If a new lesion was detected radiologically, pathological findings were used to help define whether the lesion was benign or malignant.

Primary cultures of breast cancer tissues

Tumor tissues were stored in Hank's balanced salt solution (Gibco BRL, Rockville, MD, USA) containing 100 IU/ml of penicillin (Sigma, St. Louis, MO, USA), 100 µg/ml of streptomycin (Sigma), 100 µg/ml of gentamicin (Gibco BRL), 2.5 µg/ml of amphotericin B (Gibco BRL), and 5% fetal bovine serum (Gibco BRL). When required, tissues were washed, quantitated, minced, and then incubated with a mixture of dispase, pronase, and DNase (Sigma) for 12–16 h at 37 °C. Cells were then harvested using a cell strainer (BD Falcon, Bedford, MA, USA), and to eliminate normal cells, suspensions were subjected to Ficoll (1.077 g/ml) gradient centrifugation at 400g for 15 min. Isolated cell viabilities were tested by trypan blue exclusion. Tumor tissue histological type determinations and qualitative and quantitative analyses of cancer cells were conducted by a pathologist.

Anticancer treatment and ATP-CRA

Separated tumor cells, obtained as described above, were diluted to 2000–20,000 viable cells/100 µl using IMDM (Gibco BRL) containing 10% FBS and seeded in triplicate into a 96-well ultra low attachment microplate (Costar, Cambridge, MA, USA), which restricted the growth of normal cells like fibroblasts. In the treated groups, 100 µl of the chemotherapeutic agents was added to seeded cells and cultured for 48 h in a CO₂ incubator. In control groups, 100 µl of IMDM was added to 3–6 microplate wells without chemotherapeutic agents. For quality control purposes, a negative control group of 3–6 wells (seeding medium only without cells) and two positive control groups were included in the culture plate. Each positive control group was composed of three wells that contained minimal (105 pg ATP) or median (280 pg ATP) amounts of ATP measured in 1000 tumor cells harvested from tumor tissues. The final concentrations of anticancer drugs were

determined using training set experiments, and these exhibited a scattered cell death distribution in each specimen (data not shown); epirubicin (1.2 µg/ml), 5-FU (50 µg/ml), paclitaxel (8.5 µg/ml), docetaxel (3.7 µg/ml), gemcitabine (16.9 µg/ml), vinorelbine (0.18 µg/ml), doxorubicin (1.5 µg/ml) or methotrexate (0.37 µg/ml).

The untreated control group consisted of the primary culture cells from each patient, which were cultured without any chemotherapeutic agent. The treated group consisted of the primary culture cells from each patient, which were cultured with each chemotherapeutic agent. Cells from the untreated control group and treated group were lysed, and the amount of ATP in the cell lysates were determined by luciferin and excessive luciferase (Roche, Mannheim, Germany), followed by flash-type luminescence measurements using a Victor 3 multi-label counter (PerkinElmer Boston, MA, USA). Cell death rates were calculated for each drug as follows: Cell death rate (%) = $\{1 - (\text{mean luminescence in treated group} / \text{mean luminescence in untreated control group})\} \times 100$.

To calculate the intra-assay mean coefficients of variation (CV), luminescence values of each specimen were measured 3–6 times in negative and positive control groups. We then determined whether measured values at 280 pg of ATP were higher than at 105 pg of ATP. If microorganism contamination was present, if there was an inadequate number of cells, or if the intra-assay mean CV exceeded 30, the test concerned was considered a failure. If measured values in the untreated control group were lower than in the positive group (105 pg of ATP), the specimen concerned was considered to have unacceptable viability.

Immunohistochemical assay

Expression of estrogen receptor (ER), progesterone receptor (Pg-R), p53, erb-B2, Ki67, Bcl-2, Bcl-xL, and annexin I was determined by immunostaining. The characteristics of the antibodies used in this study are summarized in Table 1. Tissues for immunohistochemical assay were obtained from paraffin blocks of tru-cut biopsy specimens taken from patients before chemotherapy. Sections were cut from each block, dewaxed in xylene, and then hydrated using graded concentrations of ethanol

in distilled water. Microwave antigen retrieval was performed before incubation with primary antibodies at room temperature for 5 min. Samples were incubated with each antibody at various dilutions (Table 1). After incubation, specimens were processed by the avidin–biotin peroxidase complex method to detect protein accumulation. Immunostaining was performed using an automated staining system (Bond-X System; Vision Biosystems, Mount Waverley, VIC, Australia). Expression of erb-B2 was categorized as 0, 1+, 2+, or 3+.²¹ Expression of other markers is reported as an expression rate. All samples were evaluated without knowledge of clinical outcomes or ATP-CRA results.

Statistical analysis

Chemotherapeutic responses of primary breast tumors were clinically evaluated as follows. Sizes of tumors were defined as length × width on radiological images. Clinical response was defined according to WHO criteria.²² CR was defined as the disappearance of all disease, and partial response (PR) as a ≥50% decrease in tumor size. No change (NC) was defined as a decrease in tumor size of <50% or an increase of <25%, and progressive disease (PD) as an increase in tumor size of ≥25% or the appearance of a new lesion. After surgery, surgical specimens were evaluated by a pathologist. Those that achieved CR or PR were defined as responders, and those that achieved NC or PD as non-responders.

Responders and non-responders were compared with regard to ATP-CRA results and biological marker expression using the Mann–Whitney and Fisher's exact tests. *P*-values of <0.05 were considered significant throughout.

Results

Clinical characteristics of patients

Forty-three patients were included in this study. All patients were women whose mean age was 46.86 ± 9.97 years (median 46.0 years, range 29–70 years), and the mean tumor size according to ultrasonography or MRI was 4.29 ± 3.05 cm (median 3.15 cm, range 1.0–14.1 cm).

Evaluability rate

We failed to culture cancer cells from three of the 43 patients. One of the samples was placed at –18 °C, another sample was stored in the wrong media. The third sample did not yield an adequate number of cells. Thus, the evaluability rate of the ATP assay using tru-cut biopsy specimens was 93.0% (40 out of 43).

In vitro drug sensitivity

The mean number of chemotherapeutic agents tested per tumor was 7.03 ± 1.41 (median 7.5, range 4–11). A list of the chemotherapeutic agents tested and their

Table 1
Antibodies used for immunohistochemistry.

Antibody	Vendor	Dilution
ER- α	Novocastra, United Kingdom	1:800
Pg-R	Novocastra, United Kingdom	1:1400
p53	Novocastra, United Kingdom	1:1500
Ki67	Novocastra, United Kingdom	1:400
erb-B2	Novocastra, United Kingdom	1:2500
Bcl-2	Novocastra, United Kingdom	1:400
Bcl-xL	R&D Systems Inc., MN, USA	1:400
Annexin I	BD Transduction Laboratories™, NY, USA	1:400

corresponding results are presented in Table 2. Sensitivity rates ranged from 0% to 86.0%. Epirubicin showed the highest mean cell death rate.

Table 2
Summary of the ATP assay for the agents tested.

	Number of patients	Cell death rate (mean \pm 2 SD, %)	Cell death rate (range, %)
Epirubicin	25	51.42 \pm 22.80	0.0–84.5
Doxorubicin	39	41.66 \pm 24.18	0.0–86.0
Paclitaxel	39	27.73 \pm 20.05	0.0–74.0
5-Fluorouracil	39	28.85 \pm 13.19	0.0–60.6
Vinorelbine	33	29.74 \pm 14.44	0.0–56.5
Docetaxel	38	18.36 \pm 13.39	0.0–44.4
Gemcitabine	29	23.94 \pm 16.25	0.0–58.1
Methotrexate	30	31.21 \pm 17.13	0.0–58.2
Cisplatin	6	25.53 \pm 10.98	7.8–38.4
Vincristine	1	23.20	–
Mytomycin C	1	19.70	–
Carboplatin	1	3.90	–
Bleomycin	1	3.60	–

Results of ATP-CRA and response to chemotherapy

Of the 40 successful assays, 20 were excluded from in vivo response analysis. Fifteen of the 40 patients changed their minds about undergoing neoadjuvant chemotherapy after first agreeing, and underwent a prompt operation without neoadjuvant chemotherapy. Two patients treated with cyclophosphamide were excluded, because ATP-CRA was not performed using this drug. In addition, the radiological methods used to evaluate response to chemotherapy in three patients differed, and these patients were also excluded.

Thus, 20 patients were evaluable for response. The clinical characteristics of these patients are shown in Table 3, which demonstrates that no statistical difference was found between the 40 patients who had the ATP-CRA performed for their tumor and the 20 patients who underwent chemotherapy.

All specimens were diagnosed as invasive ductal carcinoma NOS (not otherwise specified) by a pathologist. Nine patients (45.0%) were treated with a combination of doxorubicin and docetaxel, and 11 (55.0%) patients were managed using a combination of doxorubicin and

Table 3
Clinical characteristics of patients.

Parameter		ATP-CRA group (n = 40)	Patients received chemotherapy in the ATP-CRA group (n = 20)	P-value
Age		46.45 \pm 9.91	44.15 \pm 8.18	0.374
Stage	I	7 (17.5)	4 (20.0)	0.714
	II	14 (35.0)	8 (40.0)	
	III	7 (17.5)	5 (25.0)	
	IV	4 (10.0)	3 (15.0)	
	Unknown	8 (20.0)	0 (0.0)	
pT stage	T1	16 (40.0)	9 (45.0)	0.605
	T2	10 (25.0)	7 (35.0)	
	T3	2 (5.0)	1 (5.0)	
	T4	0 (0.0)	0 (0.0)	
	Tx	12 (30.0)	3 (15.0)	
pN stage	N0	12 (30.0)	5 (25.0)	0.760
	N1	9 (22.5)	7 (35.0)	
	N2	4 (10.0)	3 (15.0)	
	N3	3 (7.5)	2 (10.0)	
	Nx	12 (30.0)	3 (15.0)	
Estrogen receptor	Negative	22 (55.0)	13 (65.0)	0.585
	Positive	13 (32.5)	7 (35.0)	
	Unknown	1 (2.5)	0 (0.0)	
Progesterone receptor	Negative	25 (62.5)	15 (75.0)	0.558
	Positive	14 (35.0)	5 (25.0)	
	Unknown	1 (2.5)	0 (0.0)	
erb-B2	0–1+	20 (50.0)	9 (45.0)	0.669
	2+	7 (17.5)	4 (20.0)	
	3+	12 (30.0)	7 (35.0)	
	Unknown	1 (2.5)	0 (0.0)	

Table 4
Clinical characteristics of patients who underwent chemotherapy (n = 20).

Parameter	Number of patients		
	Non-responders	Responders	
Stage	I	1	3
	II	2	6
	III	2	3
	IV	1	2
pT stage	T1	3	6
	T2	2	5
	T3	0	1
	T4	0	0
	Tx	1	2
pN stage	N0	1	4
	N1	2	5
	N2	1	2
	N3	1	1
	Nx	1	2
Estrogen receptor	Negative	1	12
	Positive	5	2
Progesterone receptor	Negative	2	13
	Positive	4	1
erb-B2	0–1+	2	7
	2+	1	3
	3+	3	4

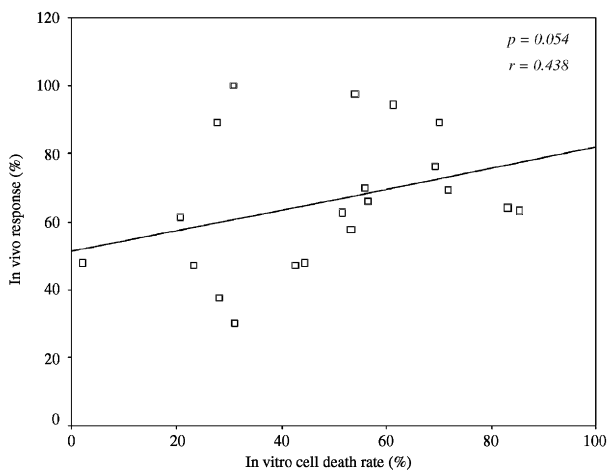


Figure 1. ATP-CRA (adenosine triphosphate-based chemotherapy response assay) results and response to chemotherapy.

paclitaxel. The clinical characteristics of non-responders and responders are shown in Table 4.

Because various chemotherapy regimens were used, we selected the drug that induced the greatest response in ATP-CRA from among the drugs used in each patient for analysis. In vitro cell death rates and in vivo response rates tended to be related ($P = 0.054$, $r = 0.438$, Figure 1).

Cell death rate as determined by ATP-CRA was significantly lower in non-responders than responders ($P = 0.012$, Figure 2). Tumors were divided into a

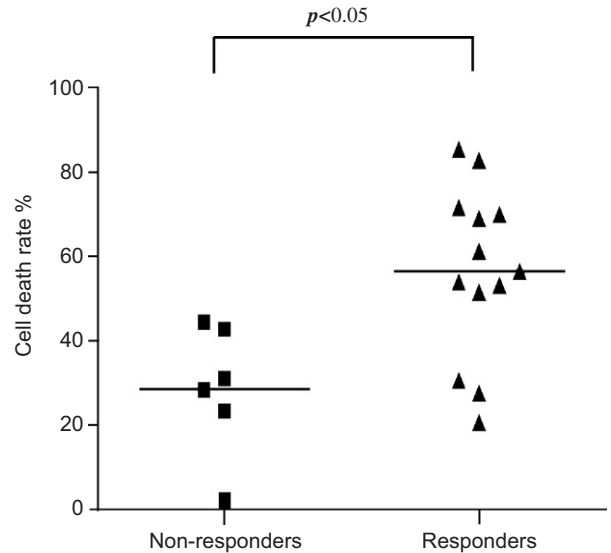


Figure 2. ATP-CRA results and in vivo response to chemotherapy. Cell death rates as determined by ATP-CRA were significantly lower in non-responders than in responders ($P < 0.05$). Bars = mean.

Table 5
ATP-CRA for predicting chemotherapeutic response in vivo.

	Responder (number)	Non-responder (number)	P-value
Sensitive group	11	0	0.002
Resistant group	3	6	

chemotherapy regimen-sensitive group and a chemotherapy regimen-resistant group using a cell death ratio cut-off of 50%. This cut-off value was selected to minimize P-values. Of the 11 tumors found to be sensitive to a drug by ATP-CRA in vitro, all 11 (100%) exhibited a response in vivo ($P < 0.05$, Table 5). Sensitivity, specificity, positive predictive value, negative predictive value, false negative value, false positive value, and diagnostic accuracy of ATP-CRA were 78.6%, 100%, 100%, 66.7%, 21.4%, 0%, and 85.0%, respectively.

Expression of various biological markers and response to chemotherapy

The expression of various biological markers (ER, Pg-R, p53, erb-B2, Ki67, Bcl-2, Bcl-xL, and annexin I), as determined by immunohistochemistry, were compared in non-responders and responders, and a significant difference was observed in the expression of ER ($P < 0.05$) and Pg-R ($P < 0.05$, Table 6). Tumors were divided into sensitive and resistant groups using a cut-off value of 10% for ER and Pg-R immunostaining results. Sensitivity, specificity, positive predictive value, negative predictive value, false negative value, false positive value, and diagnostic accuracy of ER and Pg-R immunostaining were 30.0%, 0%, 37.5%, 0%, 70%, 100% and 20%, respectively. The expression of p53, erb-B2, ki67, bcl-2, bcl-xL, and

Table 6
Immunohistochemical expression of various biological markers and response to chemotherapy.

		Non-responders (number)	Responders (number)	P-value
ER	Negative	0	7	0.026
	Positive	5	3	
Pg-R	Negative	0	7	0.026
	Positive	5	3	
erb-B2	0–1+	3	5	0.568
	2+	0	1	
	3+	2	4	

Table 7
Immunohistochemical expression rates of various biological markers and response to chemotherapy.

	Non-responders (%)	Responders (%)	P-value
ER	68.00 ± 24.90	13.00 ± 22.14	0.005
Pg-R	58.00 ± 31.14	7.00 ± 11.60	0.003
p53	16.00 ± 23.02	53.89 ± 40.14	0.083
Ki67	13.20 ± 15.39	25.50 ± 13.63	0.099
Bcl-2	65.00 ± 43.59	33.33 ± 39.37	0.330
Bcl-xL	85.50 ± 9.57	84.44 ± 11.02	0.604
Annexin I	42.50 ± 38.62	65.56 ± 31.27	0.260

annexin-I was not found to be related to tumor response to chemotherapy (Table 7).

Discussion

This study demonstrates that ATP-CRA has a high success rate, sensitivity, positive predictive value, and diagnostic accuracy. This result concurs with the findings of studies on the use of ATP-CRA in other solid cancers.^{18,20} Moreover, ATP-CRA can be performed with small amounts of cancer tissue, and specimens obtained by tru-cut biopsy are sufficient.²⁰ In the present study, all samples were taken by tru-cut biopsy, and only two or three pieces of these specimens were used for ATP-CRA. These findings are equal to or better than results achieved when samples were obtained by surgery or by vacuum-assisted core needle biopsy.⁹ The minimum sample requirement is an important factor for in vitro chemosensitivity testing because neoadjuvant chemotherapy is considered an effective and useful means of treating breast cancer. Moreover, neoadjuvant chemotherapy now plays an established role in increasing breast conservation rates in patients with large operable tumors, and a long-established history of treating locally advanced breast cancer.^{23,24} However, progression during neoadjuvant chemotherapy and the lack of an objective response portend a poor prognosis.^{4,25,26}

Recurrent tumors detected by palpation or by radiological examination may also be sampled by tru-cut biopsy.²⁰ Moreover, tru-cut biopsy is simple and cheap compared with vacuum-assisted core needle biopsy or surgical biopsy. Thus, ATP-CRA may be very useful for planning neoadjuvant chemotherapy or managing patients with recurring lesions.

In the present study, ATP-CRA was found to have a high diagnostic accuracy (85.0%) for predicting response to drugs despite the small sizes of the test samples used. Moreover, this diagnostic accuracy was equal to or superior to those of HTCA, CCS (capillary cloning system), DiSC, MTT, CD-DST (culture-drug-sensitivity test) or EDRA.^{9,27–30} This indicates that ATP-CRA may be clinically useful for deciding on chemotherapy type.

Recently, a microarray was investigated to predict response to neoadjuvant chemotherapy,^{31–34} and although some results were promising, the acceptability of the method remains controversial because of mRNA instability and difficulty with data analysis.^{35,36}

We also studied the clinical usefulness of biological markers that have frequently been studied as potential predictors of chemosensitivity to various drugs. In particular, ER, Pg-R, Ki67, erb-B2, Bcl-2, Bcl-xL, and annexin I have been used as biological markers to predict tumor response to chemotherapeutic agents and prognosis.^{8,23,37–41} However, we do not know which markers predict response to neoadjuvant chemotherapy. The present study shows that although the expression of ER or Pg-R significantly predicts tumor response to neoadjuvant chemotherapy, their diagnostic accuracy is lower than that of ATP-CRA.

Some reports have shown that neoadjuvant chemotherapy causes major changes in gene expression in breast cancer; thus, we included patients with newly diagnosed breast cancer to avoid the effect of previous chemotherapy, radiotherapy, and anti-estrogenic therapy.^{41–43} For the same reason, tru-cut biopsy specimens were taken at the time of diagnosis and these were used for immunohistochemistry studies. Patients with a tumor containing a ductal carcinoma in situ component of >80% were excluded because the intraductal component of breast cancer is known to respond poorly to neoadjuvant chemotherapy.^{44,45}

The results of in vivo responses to chemotherapeutic agents, as determined by radiography, were corrected according to pathology findings after surgery. In one case, a new lesion was observed on radiology after chemotherapy, but pathology findings showed that this lesion was benign. In another case, a radiogram showed residual tumor after neoadjuvant chemotherapy. However, this patient was revealed to have CR after surgery.

This study had several limitations. First, this experiment was performed on a small number of patients; thus, it is difficult to draw a definite conclusion about the clinical applicability of ATP-CRA. Also, the small number of patients could be the reason why biomarkers (except ER

and Pg-R) failed to show a correlation with response rate. A future study in a larger number of patients is required to confirm the clinical utility of ATP-CRA. Second, even though chemotherapy regimens involved drug combinations, ATP-CRA was evaluated for individual drugs. We are now investigating the accuracy of ATP-CRA using combination regimens. Finally, we measured the expression of erb-B2 using immunohistochemistry and not fluorescence in situ hybridization (FISH); this, too, may be a weak point of the present study.

Conclusion

Our study suggests that ATP-CRA may be used clinically to predict chemoresponse in breast cancer. Its high success in this context and its minimal sample requirements are important advantages of ATP-CRA. Moreover, prediction of response to chemotherapeutic agents by ATP-CRA was found to be superior to that achieved using the potential biological markers commonly studied. A future study in a larger patient cohort is required to confirm the clinical utility of ATP-CRA.

References

1. Ferlay J. *International Agency for Research on Cancer. GLOBO-CAN2000: cancer incidence, mortality and prevalence worldwide (monograph on CD-ROM). Version 1.0*. Lyon: IARC Press; 2001.
2. Korea Central Cancer Registry. 2002 Annual Report of the Korea Cancer Registry. Gwacheon, Republic of Korea: Ministry of Health and Welfare; 2003.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001;**94**:153–6.
4. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol* 1998;**16**:2672–85.
5. Scholl SM, Pierga JY, Asselain B, et al. Breast tumour response to primary chemotherapy predicts local and distant control as well as survival. *Eur J Cancer* 1995;**31A**:1969–75.
6. Ferriere JP, Assier I, Cure H, et al. Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol* 1998;**21**:117–20.
7. Machiavelli MR, Romero AO, Perez JE, et al. Prognostic significance of pathological response of primary tumor and metastatic axillary lymph nodes after neoadjuvant chemotherapy for locally advanced breast carcinoma. *Cancer J Sci Am* 1998;**4**:125–31.
8. Charfare H, Limongelli S, Purushotham AD. Neoadjuvant chemotherapy in breast cancer. *Br J Surg* 2005;**92**:14–23.
9. Takamura Y, Kobayashi H, Taguchi T, Motomura K, Inaji H, Noguchi S. Prediction of chemotherapeutic response by collagen gel droplet embedded culture-drug sensitivity test in human breast cancers. *Int J Cancer* 2002;**98**:450–5.
10. Kern DH, Drogemuller CR, Kennedy MC, Hildebrand-Zanki SU, Tanigawa N, Sondak VK. Development of a miniaturized, improved nucleic acid precursor incorporation assay for chemosensitivity testing of human solid tumors. *Cancer Res* 1985;**45**:5436–41.
11. Tanigawa N, Morimoto H, Dohmae N, Shimomatsuya T, Takahashi K, Muraoka R. In vitro growth ability and chemosensitivity of gastric and colorectal cancer cells assessed with the human tumour clonogenic assay and the thymidine incorporation assay. *Eur J Cancer* 1992;**28**:31–4.
12. Wilbur DW, Camacho ES, Hilliard DA, Dill PL, Weisenthal LM. Chemotherapy of non-small cell lung carcinoma guided by an in vitro drug resistance assay measuring total tumour cell kill. *Br J Cancer* 1992;**65**:27–32.
13. Mechetner E, Kyshtoobayeva A, Zonis S, et al. Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin. *Clin Cancer Res* 1998;**4**:389–98.
14. Furukawa T, Kubota T, Hoffman RM. Clinical applications of the histoculture drug response assay. *Clin Cancer Res* 1995;**1**:305–11.
15. Kang SM, Park MS, Chang J, et al. A feasibility study of adenosine triphosphate-based chemotherapy response assay as a chemosensitivity test for lung cancer. *Cancer Res Treat* 2005;**37**:223–7.
16. Maehara Y, Anai H, Tamada R, Sugimachi K. The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *Eur J Cancer Clin Oncol* 1987;**23**:273–6.
17. Cree IA, Kurbacher CM. Individualizing chemotherapy for solid tumors—is there any alternative? *Anticancer Drugs* 1997;**8**:541–8.
18. Konecny G, Crohns C, Pegram M, et al. Correlation of drug response with the ATP tumorchemosensitivity assay in primary FIGO stage III ovarian cancer. *Gynecol Oncol* 2000;**77**:258–63.
19. Kawamura H, Ikeda K, Takiyama I, Terashima M. The usefulness of the ATP assay with serum-free culture for chemosensitivity testing of gastrointestinal cancer. *Eur J Cancer* 1997;**33**:960–6.
20. Ng TY, Ngan HY, Cheng DK, Wong LC. Clinical applicability of the ATP cell viability assay as a predictor of chemoresponse in platinum-resistant epithelial ovarian cancer using nonsurgical tumor cell samples. *Gynecol Oncol* 2000;**76**:405–8.
21. Tsuda H. HER-2 (c-erb-B2) test update: present status and problems. *Breast Cancer* 2006;**13**:236–48.
22. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;**47**:207–14.
23. Shannon C, Smith I. Is there still a role for neoadjuvant therapy in breast cancer? *Crit Rev Oncol Hematol* 2003;**45**:77–90.
24. Kaufmann M, von Minckwitz G, Rody A. Preoperative (neoadjuvant) systemic treatment of breast cancer. *Breast* 2005;**14**:576–81.
25. Cameron DA, Anderson ED, Levack P, et al. Primary systemic therapy for operable breast cancer—10-year survival data after chemotherapy and hormone therapy. *Br J Cancer* 1997;**76**:1099–105.
26. Bonadonna G, Valagussa P, Brambilla C, et al. Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J Clin Oncol* 1998;**16**:93–100.
27. Xu JM, Song ST, Tang ZM, et al. Neoadjuvant chemotherapy in inoperable, locally advanced, and inflammatory breast carcinoma: a pilot study of MTT assay in vitro and outcome analysis of 10 patients. *Am J Clin Oncol* 2001;**24**:259–63.
28. Gazdar AF, Steinberg SM, Russell EK, et al. Correlation of in vitro drug-sensitivity testing results with response to chemotherapy and survival in extensive-stage small cell lung cancer: a prospective clinical trial. *J Natl Cancer Inst* 1990;**82**:117–24.
29. Von Hoff DD, Kronmal R, Salmon SE, et al. A Southwest Oncology Group study on the use of a human tumor cloning assay for predicting response in patients with ovarian cancer. *Cancer* 1991;**67**:20–7.
30. Loizzi V, Chan JK, Osann K, Cappuccini F, DiSaia PJ, Berman ML. Survival outcomes in patients with recurrent ovarian cancer who were treated with chemoresistance assay-guided chemotherapy. *Am J Obstet Gynecol* 2003;**189**:1301–7.
31. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003;**362**:362–9.
32. Cleator SJ, Powles TJ, Dexter T, et al. The effect of the stromal component of breast tumours on prediction of clinical outcome using gene expression microarray analysis. *Breast Cancer Res* 2006;**8**:R32.
33. Van Laere S, Van der Auwera I, Van den Eynden GG, et al. Distinct molecular signature of inflammatory breast cancer by cDNA microarray analysis. *Breast Cancer Res Treat* 2005;**93**:237–46.
34. Dressman HK, Hans C, Bild A, et al. Gene expression profiles of multiple breast cancer phenotypes and response to neoadjuvant chemotherapy. *Clin Cancer Res* 2006;**12**:819–26.

35. Pierga JY, Reis-Filho JS, Cleator SJ, et al. Microarray-based comparative genomic hybridization of breast cancer patients receiving neoadjuvant chemotherapy. *Br J Cancer* 2007;**96**:341–51.
36. Reis-Filho JS, Westbury C, Pierga JY. The impact of expression profiling on prognostic and predictive testing in breast cancer. *J Clin Pathol* 2006;**59**:225–31.
37. Wang Y, Serfass L, Roy MO, Wong J, Bonneau AM, Georges E. Annexin-I expression modulates drug resistance in tumor cells. *Biochem Biophys Res Commun* 2004;**314**:565–70.
38. Ahn SH, Sawada H, Ro JY, Nicolson GL. Differential expression of annexin I in human mammary ductal epithelial cells in normal and benign and malignant breast tissues. *Clin Exp Metastasis* 1997;**15**:151–6.
39. Sharma S, Neale MH, Di Nicolantonio F, et al. Outcome of ATP-based tumor chemosensitivity assay directed chemotherapy in heavily pre-treated recurrent ovarian carcinoma. *BMC Cancer* 2003;**3**:19.
40. Ogston KN, Miller ID, Schofield AC, et al. Can patients' likelihood of benefiting from primary chemotherapy for breast cancer be predicted before commencement of treatment? *Breast Cancer Res Treat* 2004;**86**:181–9.
41. Hannemann J, Oosterkamp HM, Bosch CA, et al. Changes in gene expression associated with response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2005;**23**:3331–42.
42. Dowsett M, Smith IE, Ebbs SR, et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. *Clin Cancer Res* 2005;**11**:951s–8s.
43. Piper GL, Patel NA, Patel JA, Malay MB, Julian TB. Neoadjuvant chemotherapy for locally advanced breast cancer results in alterations in preoperative tumor marker status. *Am Surg* 2004;**70**:1103–6.
44. Wu W, Kamma H, Ueno E, et al. The intraductal component of breast cancer is poorly responsive to neo-adjuvant chemotherapy. *Oncol Rep* 2002;**9**:1027–31.
45. Matsuo K, Fukutomi T, Watanabe T, Hasegawa T, Tsuda H, Akashi-Tanaka S. Concordance in pathological response to neoadjuvant chemotherapy between invasive and noninvasive components of primary breast carcinomas. *Breast Cancer* 2002;**9**:75–81.