Chemosensitivity of Breast Cancer Lymph Node Metastasis Compared to the Primary Tumor from Individual Patients Tested in the Histoculture Drug Response Assay

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Abstract. *Lymph node metastasis is often the first indication of the aggressiveness of breast cancer. Effective chemotherapy in breast cancer depends on targeting the metastatic component of the disease. In order to optimize chemotherapy in the metastatic target of breast cancer, the histoculture drug response assay (HDRA) was performed on surgical specimens of primary tumor and axillary lymph node metastasis from 30 breast cancer patients. The surgical specimens were cut into approximately 10 mg pieces, and placed onto the collagen gel sponges in the medium containing previously-determined cutoff concentrations of doxorubicin (DXR), 5-fluorouracil (5-FU), cisplatin (DDP), and mitomycin C (MMC). After incubation for 7 days, the chemosensitivity of the tumor fragments was evaluated with the 3-(4,5-dimethythiazol2yl) -2,5-diphenyl-2H tetrazolium bromide (MTT) endpoint. The lymph node metastases were more resistant than the primary tumor for DXR, 5-FU, and MMC ($p<0.05$) but not for CDDP. The data suggest that both primary tumor and metastases from individual patients should be tested in the HDRA to enhance clinical efficacy of chemotherapy.*

Hoffman *et al.* (1-6) have developed the histoculture drug response assay (HDRA), which allows the tumor specimens to maintain cell-to-cell contact in their native three-dimensional tissue architecture enabling accurate assessment of clinical chemosensitivity. A high correlation of chemotherapy and survival for gastric and colorectal cancer patients was obtained using the HDRA (3-5).

Since tumors are heterogeneous, there may be a difference in chemosensitivity between primary and metastatic lesions. In the present study, we have compared the *in vitro* chemosensitivity of primary tumor and axillary lymph node metastasis obtained from individual breast cancer patients.

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Materials and Methods

Drugs. Doxorubicin (DXR), 5-fluorouracil (5-FU), and mitomycin C (MMC) were purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Cisplatin (DDP) was purchased from Bristol- Meyers Squibb, Tokyo, Japan.

Histoculture drug response assay (HDRA). Specimens of primary tumor and axillary lymph node metastasis were obtained from 30 patients with breast cancer in single surgical procedures at the Department of Thoracic and Cardiovascular Surgery, and Department of Surgery, Kihoku Hospital, Wakayama Medical College. Test samples of the surgical specimens were transported to the laboratory in Hanks' balanced salt solution (HBSS; GIBCO, Gaithersburg, MD).

The HDRA was performed according to previously reported methods (3-6). In brief, the chemotherapeutic drugs were dissolved in RPMI 1640 medium (Sigma, St. Louis, MO) containing 20% fetal calf serum (IANSA, Mexico), penicillin-streptomycin-amphotericin B (GIBCO, 100 μ/ml, 100 μ/ml, and 0.25 μ/ml, respectively). One ml per well of the solution was poured into 24-well plates. Six and four replicates were run for control and treatment groups, respectively.

The drug concentration used were 15 μl/ml for DXR, 300 μl/ml for 5-FU, 2 μl/mlfor MMC, and 20 μl/ml for CDDP. The collagen gel sponge (Gel Foam R; Pharmacia & Upjohn, Inc., UK) was cut into 1 cm^3 pieces and placed into the wells of the plates. The surgical specimens were cut into approximately 10 mg pieces, weighed with a balance (R200D, Sartorius, Germany), and placed onto the collagen gel sponges. Histocultures were incubated in 5% CO_2 at 37° C for 7 days.

After the culture period, 100 μl of 0.06% collagenase (type I; Sigma, St. Louis, MO) solution in HBSS, and 100 μl of 0.2% MTT (Sigma) phosphate buffer saline (PBS) solution containing 50 mM sodium succinate (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) were added to each well. After the plates were incubated for 16 hours, the medium was removed and 0.5 ml dimethyl sulfoxide (DMSO) was added to each well to extract the MTT-formazan. After 2 hours, 100 μl of extract solution of each well was transferred to 96 well plates and the absorbance determined at 540 nm. The inhibition rate (I.R) (%) = (2 - A/B) x 100, where A is the mean absorbance of the, treated wells per lg tumor and B is the mean absorbance of the control wells per 1g tumor.

The difference in the inhibition rates (ΔI.R.), in absolute values, of the primary and metastatic tumors was calculated for each patient and drug.

Table I. *Chemosensitivity of primary and metastatic lesions of breast cancer from individual patients in the HDRA.*

Drugs	Primary lesion M±SD	Primary lesion Range	Lymph node metastasis M±SD	Lymph node metastasis Range	Δ I.R.[a] M±SD	Δ I.R.[a] Range
DXR	61.4 ± 17.5[b]	6.5 ~ 78.8[c]	42.6 ± 27.3*	0 ~ 27.4	23.9 ± 21.3	2.0 ~ 69.3
5-FU	46.4±18.5	6.9 ~ 72.9	32.2±23.5*	0 ~ 78.3	25.1±18.3	2.6 ~ 57.4
MMC	66.7±18.8	0 ~ 84.4	52.6±27.6*	0 ~ 8_.4	21.2±21.4	0.9 ~ 66.1
DDP	37.9 ± 14.5	11.3 ~ 66.6	31.0 ± 20.1	0 ~ 58.8	16.6 ± 12.5	0.9 ~ 46.8
Total	53.1 ± 20.6	0 ~ 88.4	39.5 ± 26.0	0 ~ 8_.4	21.7± 12.5	0.9 ~ 69.3

Abbreviations used are: DXR, doxorubicin, 5-FU, 5-fluorouracil, MMC, mitomycin C, DDP, cisplatin.
[a] ΔI.R. = inhibition rate of primary tumor - inhibition rate of metastatic lymph node.
[b] Date were shown as inhibition rate (%) (mean ± standard deviation).
[c] Data were shown as inhibition rate in %.
* $p<0.05$ relative to primary lesion by Student's t-test.

Results and Discussion

All the specimens were evaluable with the HDRA. The comparison of inhibition rates of primary tumor and lymph node metastasis is shown in Table I. The chemosensitivity, as indicated by drug inhibition rates, of the primary tumors was higher than that of the lymph node metastases with a statistically significant difference at $p<0.05$ for DXR, 5-FU and MMC but not for CDDP. The means of the difference in the inhibition rates (ΔI.R.s) were approximately 20%.

It is well known that the malignant tumor consists of heterogeneous clones of cancer cells that metastasize to other organs. This suggests that the drug sensitivity of metastatic lesions might be different from those of primary lesion.

However, Kerbel *et al* (10) reported that metastases are derived from the dominant clone population of the primary tumors. In their experiments, genetically-marked cell clones were injected into mice. The primary tumor was removed about 6-7 weeks later along with resulting lung metastases. When the tumors were analyzed by Southern blotting, they were found to be essentially composed of the progeny of a single clone. This suggested that spontaneous metastases developed in a non-random fashion from genotypically distinct cell clones.

In the present study, we have compared the sensitivity of primary tumor and axillary lymph node metastases in the same patients with breast cancer, indicating that the lymph node metastases were more resistant than the primary tumor for DXR, 5-FU, and MMC but not CDDP. (Table I). The data in this report suggest that both primary and metastatic lesions should thus be tested, whenever possible, in the HDRA to ensure maximum clinical efficacy of drugs found effective in the HDRA.

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