Tumori, 96: 424-432, 2010

Metastatic breast cancer shows different immunohistochemical phenotype according to metastatic site

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ABSTRACT

Aims and background. The study was performed to assess the status of immunohistochemical markers in primary and metastatic breast cancer and to determine the organ-specific characteristics of metastatic breast cancer.

Methods. Samples from 13 cases of paired primary and metastatic breast cancer and 34 cases of metastatic breast cancer were included.

Results. In the analysis of 13 cases of paired primary and metastatic breast cancer, estrogen receptor and progesterone receptor loss were noted in 1 (7.7%) case each. Androgen receptor loss and gain was noted in 2 (15.4%) cases, respectively. HER-2 showed 100% concordance with primary and metastatic tumors. C-kit was demonstrated in only 2 (15.4%) cases of metastatic breast cancer. In the analysis of 34 cases of metastatic breast cancer, when classified into triple-negative type (ER-, PR-, and HER-2-), HER-2+ type, and ER+ or PR+/HER-2- type according to immunohistochemical stain results, HER-2 type (66.7%) in brain metastasis and ER+ or PR+/HER-2- type (75.0%) in liver metastasis were predominant. Bone metastasis was composed of triple negative type (44.4%) and ER+ or PR+/HER-2- type (55.6%), and lung metastasis showed all of three subtypes in similar proportions.

Conclusions. Metastatic breast cancer shows different immunohistochemical phenotypes according to metastatic site ($P = 0.048$). Free full text available at www.tumorionline.it

Introduction

Breast cancer is one of the diseases with the highest morbidity and mortality worldwide, which is partly due to distant metastasis. The usual sites of distant metastasis of breast cancer are the lung, brain, liver, and bone[1]. The general mechanism of tumor metastasis is composed of reciprocal interactions between tumor cells and host tissues, which include alterations in adhesion, proteolysis, invasion, and angiogenesis[2,3]. However, not all tumors show similar patterns of metastasis, which led to development of the seed-and-soil hypothesis[4]. The theory suggests that a specific tumor (seed) can survive in only a specific visceral organ (soil) that can give nutrients to tumor cells. It has provided a conceptual framework for understanding the clinical behavior and pattern of solid tumors such as breast cancer.

The present treatment modalities for metastatic tumors are conventional chemotherapy, hormone therapy, targeted biological agents, and radiation therapy. Whichever method is applied, the main target is metastatic foci[5]. However, the aforementioned treatments are conducted in the understanding that the genotype of metastatic breast cancer is similar to primary breast cancer. Meanwhile, many experiments and research have suggested the following: 1) the primary tumor is genetically heterogeneous, 2) the clones that lead to organ dissemination may not even be present in the primary tumor but develop through sequential genetic alteration, and

Key words: breast neoplasm, cancer metastasis, immunohistochemistry.

Acknowledgments: The present research was supported by the Korean Breast Cancer Foundation.

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Received August 31, 2009;
accepted January 13, 2010.

3) they make up a very small percentage of the primary tumor[6]. Therefore, to develop a precise, targeted therapy for metastatic tumors, the specific features of metastatic tumors, which are different from primary tumors, must be evaluated.

Studies of metastatic breast cancer have mainly focused on brain and bone metastasis[7-12], and few data about the specific characteristics of metastatic breast cancer according to metastatic organ are available. If organ-specific characteristics of metastatic breast cancer could be identified, they would be helpful in understanding the nature of metastatic breast cancer. If the metastatic tumor marker could be determined, target therapy for this marker would be created and outcomes would improve.

The purpose of the present study was to assess the status of immunohistochemical markers in primary breast cancer *versus* metastatic breast cancer and to determine the organ-specific characteristics of metastatic breast cancer.

Materials and methods

Patient selection

From the files of the Department of Pathology of Severance Hospital and Yongdong Severance Hospital, tissue samples from female patients with invasive primary breast cancer and metastasis to distant organs (liver, lung, brain, and bone) were retrieved. Only patients with a diagnosis of invasive ductal carcinoma were included. The study was approved by the Institutional Review Board. Formalin-fixed and paraffin-embedded tissue specimens from 34 cases of metastatic tumors (lung, liver, brain, and bone) were used. Thirteen of the 34 cases had corresponding primary breast cancer tissue. We could not obtain corresponding primary breast cancer tissue for 21 cases of metastatic tumor because 21 patients were treated for the primary breast cancer in other hospitals. The mean age of the 34 patients was 42 years (range, 28-60). The mean follow-up was 79 months (range, 20-243). The treatment modality was surgery + chemoradiotherapy in 15 (44.1%) cases, surgery + chemotherapy in 7 (20.6%) cases, surgery only in 7 (20.6%) cases, and neoadjuvant chemotherapy + surgery + chemoradiotherapy in 5 (14.7%) cases. All slides were reviewed again, and pathologic diagnoses were approved by two pathologists (JSK and WJ).

Tissue microarray

On hematoxylin-eosin-stained slides of tumors, a representative area was selected and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out and a 3-mm tissue core was placed into a 5 x 6 recipient block. More than 2 tissue cores were extracted to minimize extraction bias. Each tissue core was assigned a unique tissue microarray location number that was linked to a data base containing other clinicopathologic data.

Immunohistochemistry

The antibodies used for immunohistochemistry in the study are shown in Table 1. All immunostainings were performed using formalin-fixed, paraffin-embedded tissue sections. Five-μm-thick sections were obtained with a microtome, transferred to adhesive slides, and dried at 62 °C for 30 min. After incubation with primary antibodies, immunodetection was performed with biotinylated antimouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3 -diaminobenzidine chromogen as substrate. Optimal primary antibody incubation time and concentration were determined via serial dilution for each immunohistochemical assay with an identically fixed and embedded tissue block. Slides were counterstained with Harris hematoxylin. The staining was interpreted by two pathologists (JSK and WJ) under a multiview microscope. Different results were discussed, and in case of persistent discordance, a third pathologist was consulted.

All immunohistochemical markers were assessed by light microscopy. Scoring of immunostained slides was done according to the percentage of tumor cells exhibiting nuclear (androgen receptor [AR], estrogen receptor [ER], progesterone receptor [PR], and p53), nuclear and cytoplasmic (c-kit, vascular endothelial growth factor [VEGF]), cytoplasmic (cytokeratin [CK5], vimentin, and bcl-2), and membrane (E-cadherin, epithelial growth factor receptor [EGFR], and HER-2) staining. Expression of CD10 was evaluated in peritumoral stromal cells showing cytoplasmic staining. ER and PR immunohistochemistry signal was evaluated using the Allred

Table 1 - Clone, dilution, and source of antibodies used

Antibody	Clone	Dilution	Source
ER	6FH	1:100	Novocastra, UK
PR	1A6	1:200	Novocastra, UK
AR	AR441	1:100	Lab Vision Corp.
CK5	XM26	1:100	Novocastra, UK
E-cadherin	36B5	1:100	Novocastra, UK
P53	DO-7	1:100	Novocastra, UK
EGFR	EGFR.25	1:50	Novocastra, UK
Bcl-2	3.1	1:50	Novocastra, UK
c-kit	Polyclonal	1:100	DAKO, Denmark
Vimentin	V9	1:150	DAKO, Denmark
HER-2	c-erbB-2	1:100	DAKO, Denmark
CD10	56C6	1:100	Novocastra, UK
VEGF	Polyclonal	1:250	Santa Cruz, CA

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; CK5, cytokeratin 5; EGFR, epithelial growth factor receptor; VEGF, vascular endothelial growth factor.

score[13]. Briefly, a proportion score was assigned representing the estimated proportion of positive-staining tumor cells (0 = none, 1 = <1/100, 2 = 1/100 to <1/10, 3 = 1/10 to <1/3, 4 = 1/3–2/3, and 5 = >2/3). The average estimated intensity of staining in positive cells was assigned an intensity score (0 = none, 1 = weak, 2 = intermediate, and 3 = strong). Proportion and intensity scores were added to obtain a total score that ranged from 0 to 8. A score of 0 to 2 was considered negative and 3 to 8 was considered positive. HER-2 staining was analyzed according to the American Society of Clinical Oncology /College of American Pathologists guidelines[14] using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining, less than 10% of tumor cells; 2+ = complete membranous staining, either uniform or weak in at least 10% of tumor cells; and 3+ = uniform intense membranous staining in at least 30% of tumor cells. Her2/neu immunostaining was considered positive when strong (3^+) membranous staining was observed, whereas cases with 0 to 2^+ were regarded as negative. Immunohistochemical stain results of E-cadherin, VEGF, and EGFR were scored on a 0 to 3 intensity scale (0 = negative staining, 1 = weak staining, 2 = moderately intense staining, and 3 = strong staining). Immunohistochemical stain results of AR, p53, CD10, c-kit, CK5, vimentin, and bcl-2 were considered positive when more than 10% of tumor cells were stained.

Statistical analysis

Data were statistically processed using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Correlation analysis of immunostaining results between primary breast cancer and metastatic breast cancer were calculated by the Wilcoxon test. Comparative statistics were performed using chi-square analysis. Statistical significance was assumed when P <0.05. Kaplan-Meier survival curves and logrank statistics were employed to evaluate time to tumor metastasis and time to survival. Multivariate regression analysis was performed using Cox proportional hazards model.

Results

Clinicopathologic and immunohistochemical characteristics of 34 cases of distant metastatic tumor according to metastatic organ

Table 2 demonstrates the clinicopathologic and immunohistochemical characteristics of metastatic breast cancer in 34 patients according to metastatic organ. Metastatic organ sites were the brain in 6 cases, lung in 15, liver in 4, and bone in 9. Nuclear and histologic grade revealed no significant difference among metastatic organs (P = 0.093 and 0.140, respectively). Metastatic tumors showed a high incidence of negative

Table 2 - Clinicopathologic and immunohistochemical characteristics of breast cancer according to metastatic organ

Parameter	Total (n = 34) (%)	Brain (n = 6) (%)	Lung (n = 15) (%)	Liver (n = 4) (%)	Bone (n = 9) (%)	*P*
Age,yr, mean	43	47	44	37	41	0.321
TTM, mo, mean	40	18	56	28	34	0.150
Nuclear grade						0.093
1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
2	18 (52.9)	1 (16.7)	7 (46.7)	3 (75.0)	7 (77.8)	
3	16 (47.1)	5 (83.3)	8 (53.3)	1 (25.0)	2 (22.2)	
Histologic grade						
0.140						
I	1 (2.9)	0 (0)	0 (0)	1 (25.0)	0 (0)	
II	19 (55.9)	2 (33.3)	9 (60.0)	2 (50.0)	6 (66.7)	
III	14 (41.2)	4 (66.7)	6 (40.0)	1 (25.0)	3 (33.3)	
ER						0.666
Positive	12 (35.3)	1 (16.7)	6 (40.0)	1 (25.0)	4 (44.4)	
Negative	22 (64.7)	5 (83.3)	9 (60.0)	3 (75.0)	5 (55.6)	
PR						0.321
Positive	8 (23.5)	0 (0)	4 (26.7)	2 (50.0)	2 (22.2)	
Negative	26 (76.5)	6 (100)	11 (73.3)	2 (50.0)	7 (77.8)	
AR						0.181
Positive	8 (23.5)	0 (0)	6 (40.0)	1 (25.0)	1 (11.1)	
Negative	26 (76.5)	6 (100)	9 (60.0)	3 (75.0)	8 (88.9)	
HER-2						0.016
Positive	8 (23.5)	4 (66.7)	4 (26.7)	0 (0)	0 (0)	
Negative	26 (76.5)	2 (33.3)	11 (73.3)	4 (100)	9 (100)	
CK5						
Positive	6 (17.6)	1 (16.7)	5 (33.3)	0 (0)	0 (0)	0.149
Negative	28 (82.4)	5 (83.3)	10 (66.7)	4 (100)	9 (100)	
p53						0.265
Positive	5 (14.7)	1 (16.7)	4 (26.7)	0 (0)	0 (0)	
Negative	29 (85.3)	5 (83.3)	11 (73.3)	4 (100)	9 (100)	
E-cadherin						0.506
3	6 (17.6)	2 (33.3)	2 (13.3)	0 (0)	2 (22.2)	
2	21 (61.8)	4 (66.7)	9 (60.0)	3 (75.0)	5 (55.6)	
1	3 (8.8)	0 (0)	3 (20.0)	0 (0)	0 (0)	
0	4 (11.8)	0 (0)	1 (6.7)	1 (25.0)	2 (22.2)	
Bcl-2						0.287
Positive	13 (38.2)	1 (16.7)	5 (33.3)	3 (75.0)	4 (44.4)	
Negative	21 (61.8)	5 (83.3)	10 (66.7)	1 (25.0)	5 (55.6)	
Vimentin						0.187
Positive	9 (26.5)	2 (33.3)	6 (40.0)	1 (25.0)	0 (0)	
Negative	25 (73.5)	4 (66.7)	9 (60.0)	3 (75.0)	9 (100)	
EGFR						0.383
3	2 (5.9)	0 (0)	2 (13.3)	0 (0)	0 (0)	
2	2 (5.9)	0 (0)	2 (13.3)	0 (0)	0 (0)	
1	4 (11.8)	1 (16.7)	3 (20.0)	0 (0)	0 (0)	
0	26 (76.5)	5 (83.3)	8 (53.3)	4 (100)	9 (100)	
CD10						
Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Negative	34 (100)	6 (100)	15 (100)	4 (100)	9 (100)	
VEGF						0.117
3	4 (11.8)	0 (0)	1 (6.7)	0 (0)	3 (33.3)	
2	10 (29.4)	4 (66.7)	3 (20.0)	2 (50.0)	1 (11.1)	
1	10 (29.4)	2 (33.3)	6 (40.0)	1 (25.0)	1 (11.1)	
0	10 (29.4)	0 (0)	5 (33.3)	1 (25.0)	4 (44.4)	
c-kit						0.415
Positive	4 (11.8)	1 (16.7)	3 (20.0)	0 (0)	0 (0)	
Negative	30 (88.2)	5 (83.3)	12 (80.0)	4 (100)	9 (100)	
IHC subtype						0.048
ER-/PR-/ HER-2-	13 (38.2)	1 (16.7)	5 (33.3)	1 (25.0)	4 (44.4)	
HER-2+	8 (23.5)	4 (66.7)	4 (26.7)	0 (0)	0 (0)	
ER or PR+/ HER-2-	13 (38.2)	1 (16.7)	6 (40.0)	3 (75.0)	5 (55.6)	

TTM, time to metastasis; ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; CK5, cytokeratin 5; EGFR, epithelial growth factor receptor; VEGF, vascular endothelial growth factor; IHC, immunohistochemistry.

expression in hormone receptors such as ER, PR, and AR (64.7%, 76.5%, and 76.5%, respectively). There was a trend of metastatic tumors with ER expression to be frequently observed in the lung (40.0%) and bone (44.4%) without statistical significance (P = 0.666). Metastatic breast cancer in the brain showed a distinctive trend for negative expression of hormone receptors such as ER, PR, and AR (83.3%, 100%, and 100%, respectively). HER-2 status was different among metastatic organs (P = 0.016). None of the metastatic breast cancers in the liver and bone showed HER-2 overexpression, and metastatic breast cancers in the lung showed a high incidence of negative HER-2 status (73.3%). However, metastatic breast cancers in the brain showed a high incidence of HER-2 overexpression (66.7%). CK5 and p53 were not expressed in any metastatic breast cancer in the liver and bone, and metastatic breast cancer in the brain and lung showed a trend of a negative reaction to CK5 and p53 (83.3% and 66.7% in CK5 and 83.3% and 73.3% in p53, respectively). Bcl-2 was well expressed in metastatic tumors in the liver (75.0%) but expression of bcl-2 was low in the brain (16.7%) and lung (33.3%), without statistical significance (P = 0.287). EGFR was not expressed in metastatic breast cancer of the liver or bone, and only 1 (16.7%) case of metastatic breast cancer in the brain showed EGFR expression. However, 7 (46.6%) cases of metastatic breast cancer in the lung expressed EGFR (P = 0.383). CD10 was not expressed in metastatic tumor stroma. C-kit was expressed in only 1 (16.7%) case and 3 (20.0%) cases of metastatic breast cancer in the brain and lung, respectively. E-cadherin and VEGF showed variable expression patterns according to metastatic organs without statistical significance (P = 0.506 and 0.117, respectively). When metastatic breast cancers were classified into triple negative type (ER- and PR-/HER-2-), HER-2+ type, and ER+ or PR+/HER-2- type according to immunohistochemical stain results, metastatic breast cancer showed different immunohistochemical subtypes according to metastatic site (P = 0.048). HER-2+ type (66.7%) in the brain and ER+ or PR+/HER-2- type (75.0%) in the liver were predominant. Metastatic breast cancer in the bone showed triple negative type (44.4%) and ER+ or PR+/HER-2- type (55.6%) without HER-2+ type. Metastatic breast cancer in the lung demonstrated all 3 types in similar percentages.

Clinicopathologic and immunohistochemical characteristics of 13 cases of primary breast cancer and corresponding metastatic tumor

Table 3 shows the clinicopathologic and immunohistochemical data of 13 patients with primary and corresponding metastatic breast cancer. Patient age ranged from 28 to 52 years (mean, 39). Metastatic organ sites were 3 cases in the brain, 8 in the lung, and 1 in the liver and bone. Lymph node metastasis was observed in 8 cases. Time to metastasis was 24.6 ± 28.8 months (mean ± SD; range, 3-117). Nuclear grade of primary breast cancer was 1 in 1 case, 2 in 3 cases, and 3 in 10 cases. A change of nuclear grade in metastatic breast cancer was noted in 3 cases (case nos. 6, 10, and 13) (P = 0.564). Histologic grade of primary breast cancer was I in 1 case, II in 8 cases, and III in 4 cases. A change of histologic grade in metastatic breast cancer was noted in 6 cases (P = 0.317). ER was expressed in 3 cases in primary tumors and loss of ER was noted in 1 case (case no. 13) with 92.3% concordance between ER status of the primary and metastatic tumor (P = 0.317). PR also represented 92.3% concordance between PR status of the primary and metastatic tumor as ER, but PR loss in metastatic tumor was noted in case no. 6 (P = 0.157). AR was expressed in 2 cases of primary tumors, which showed no expression in metastatic tumor. In contrast, 2 cases showing AR expression in metastatic tumors demonstrated no expression in primary tumors, resulting in a concordance rate between AR status of the primary and metastatic tumor of 69.2% (P = 0.564). HER-2, CK5, p53, and bcl-2 showed 100% concordance between primary and metastatic tumors. HER-2 was overexpressed in 3 cases. CK5, p53, and bcl-2 were expressed in 5, 4, and 3 cases, respectively. E-cadherin, EGFR, and VEGF showed variable and inconsistent immunoreactivity between primary and metastatic tumors. Vimentin was expressed in 4 cases in primary tumors. Loss and gain of vimentin in metastatic tumors were observed in 1 and 2 cases, respectively, with 76.9% concordance between primary and metastatic tumors (P = 0.564). Three cases of primary tumors showed CD10 expression in tumor stromal cells, but none expressed CD10 in metastatic tumors (P = 0.083). C-kit was expressed in only 2 cases in metastatic tumors.

Effects of clinicopathologic and immunologic parameters in metastatic tumor on time to tumor metastasis and time to overall survival

Table 4 shows univariate analyses of the effects of clinicopathologic factors and immunohistochemical markers identified in metastatic breast cancer of 34 patients on time to metastasis-free survival and overall survival. The results of univariate analyses of clinicopathologic factors and immunohistochemical markers on time to tumor metastasis revealed a statistical significance in only three parameters, ER (P = 0.037), AR (Figure 1, P = 0.020) and Bcl-2 (P = 0.031). Namely, metastatic breast cancer without expression of ER, AR, or Bcl-2 showed longer metastasis-free survival than metastatic breast cancer with expression of ER, AR, or Bcl-2. Multivariate Cox regression analysis showed a statistical significance only for AR (P = 0.001).

The results of univariate analyses of the effects of clinicopathologic factors and immunohistochemical markers on time to overall survival revealed significance

Table 3 - Clinicopathologic and immunohistochemical characteristics of paired primary and metastatic breast cancer

Case no.	Age, yr	Nuclear grade (P/M)	Histologic grade (P/M)	Lymph node metastasis	TTM (mo)	Metastatic organ	ER (P/M)	PR (P/M)	AR (P/M)	HER2 (P/M)	CK5 (P/M)	p53 (P/M)	Bcl-2 (P/M)	E-cadherin (P/M)	Vimentin (P/M)	EGFR (P/M)	CD10 (P/M)	VEGF (P/M)	c-kit (P/M)
1	46	3/3	2/2	Yes	5	Brain	-/-	-/-	-/-	-/-	+/+	+/+	-/-	1/2	-/+	1/1	-/-	1/1	-/+
2	47	3/3	3/2	No	25	Lung	-/-	-/-	-/-	-/-	+/+	+/+	-/-	1/0	+/+	2/1	+/-	1/0	-/-
3	35	3/3	3/3	Yes	22	Lung	-/-	-/-	-/-	-/-	+/+	+/+	-/-	1/1	-/+	0/1	+/-	3/3	-/-
4	28	2/2	2/2	Yes	117	Lung	+/+	+/+	-/+	-/-	-/-	-/-	+/+	2/2	-/-	0/0	-/-	0/2	-/-
5	33	3/3	3/3	Yes	11	Lung	-/-	-/-	-/-	-/-	+/+	-/-	-/-	1/2	+/+	2/3	-/-	0/0	-/-
6	38	3/2	2/1		3	Liver	+/+	+/-	+/-	-/-	-/-	-/-	+/+	3/2	-/-	0/0	-/-	3/2	-/-
7	52	3/3	2/3	Yes	25	Brain	-/-	-/-	-/-	+/+	-/-	-/-	-/-	2/3	-/-	0/0	-/-	2/2	-/-
8	42	3/3	3/3	Yes	15	Brain	-/-	-/-	-/-	+/+	-/-	-/-	-/-	1/2	-/-	0/0	-/-	2/2	-/-
9	34	3/3	2/3	Yes	23	Bone	-/-	-/-	-/-	-/-	-/-	-/-	-/-	2/2	-/-	0/0	-/-	2/3	-/-
10	47	2/3	2/3	Yes	23	Lung	-/-	-/-	+/-	+/+	-/-	+/+	-/-	2/1	-/-	0/0	+/-	2/1	-/-
11	35	2/2	2/2		11	Lung	-/-	-/-	-/-	-/-	-/-	-/-	-/-	0/2	+/-	1/2	-/-	2/0	-/-
12	44	3/3	2/2	No	14	Lung	-/-	-/-	-/-	-/-	+/+	-/-	-/-	2/2	+/+	1/2	-/-	2/1	-/+
13	32	1/2	1/3	No	26	Lung	+/-	+/+	-/+	-/-	-/-	-/-	+/+	2/2	-/-	0/0	-/-	1/1	-/-

TTM, time to metastasis; P, primary breast cancer; M, metastatic breast cancer.

of five parameters: nuclear grade (P = 0.005), PR (P = 0.012), AR (P = 0.015), Bcl-2 (P = 0.036), and organ where tumor metastasis occurred (Figure 2, P = 0.000). Metastatic breast cancer showing low nuclear grade, PR expression, AR expression, or Bcl-2 expression demonstrated longer overall survival. Longer overall survival was noted in metastatic organ in the following order; lung > liver > bone > brain. In multivariate Cox regression analysis, metastatic organ (P = 0.000) and nuclear grade (P = 0.005) showed statistical significance.

Discussion

The treatment of breast cancer has improved due to the development of new drugs and modalities. However, breast cancer is the leading cause of death for women in the world, which is partly due to distant metastasis from breast cancer[1,3]. The major organs that have metastasis from breast cancer are the brain, lung, liver, and bone[1,3]. Metastatic breast cancer is managed by medical rather than surgical treatment. Targeted therapy such as tamoxifen for ER- and PR-expressing breast cancer and trastuzumab for HER-2-overexpressing breast cancer is used according to the expressed marker of breast cancer. Therefore, expression status of these markers is important for treatment when breast cancer metastasis occurs.

In the present study, we investigated immunohistochemical markers in paired primary and metastatic breast cancers and characteristics of immunohistochemical markers in metastatic breast cancer according to metastatic organ.

In the analysis of 13 cases of paired primary and metastatic breast cancer, ER and PR were expressed in the same 3 cases. However, ER and PR loss were noted in 1 (7.7%) case each. In metastatic breast cancer, the reported ER loss ranged from 3.2 to 44%[15-17] and PR loss was 24%[17]. The difference in reported ratios was explained by different methods of evaluation for ER and PR, type of antibody, and applied positive criteria[16]. Loss of ER and PR is a well-documented phenomena, but ER and PR gain has not been reported. AR loss and gain was noted in 2 (15.4%) cases, respectively. AR gain and loss has not been as thoroughly investigated as ER and PR loss. The role of AR in breast cancer in breast carcinogenesis and progression is still uncertain, but there are divergent data about the biological and clinical significance of AR in breast cancer. In ER-negative tumors, AR-positive cases exhibited significantly better disease-free survival than AR-negative tumors[18]. Loss of expression of AR is also associated with transformation from *in situ* to invasive ductal carcinoma of the basal type[19]. However, there was a report that breast cancer with the AR gene or AR protein showed an increased tendency of axillary lymph node metastasis[20].

In the present study, HER-2 showed 100% concordance between primary and metastatic tumors, which is consistent with previous reports that HER-2 status was concordant in lymph node, liver, and lung metastasis[21,22]. However, other studies have shown that the loss of HER-2 amplification ranged from 21-50%[12,23], and HER-2 amplification was noted in visceral metastasis (30%). Of 13 cases with paired primary and metastatic breast cancer, the triple negative type was in 53.8% and ER+ or PR+/HER-2- type and HER-2+ type were both in 23.0%. This result concurs with the finding that triple-negative cancer shows an increased incidence of distant metastasis[24]. When immunohistochemical stains of CK5, vimentin, and EGFR (which are known to express in triple-negative breast cancer[25]) were performed, these markers were expressed only in triple-negative cancer. C-kit was not expressed in primary breast cancer but was demonstrated in 2 (15.4%) cases of metastatic breast cancer. In general, c-kit was strongly expressed in normal breast epithelium. However, expres-

Table 4 - Univariate analysis of effect of various clinopathologic and immunohistochemical factors in metastatic tumor of 34 patients on time to metastasis-free survival and overall survival by the logrank test

Parameter	No. of patients (n = 34) (%)		Metastasis-free survival		Overall survival	
	No. of cases	Patient death	Median survival (95% CI) mo	*P*	Median survival (95% CI) mo	*P*
Nuclear grade				0.970		0.005
2	18	8 (44.4)	35 (14-56)		149 (102-195)	
3	16	13 (81.3)	23 (20-26)		37 (33-41)	
Histologic grade				0.134		0.137
I	1	1 (100.0)	3 (n/a)		60 (n/a)	
II	19	10 (52.6)	36 (18-54)		97 (42-152)	
III	14	10 (71.4)	24 (22-26)		48 (27-69)	
ER				0.037		0.070
Positive	12	6 (50.0)	59 (45-73)		108 (94-201)	
Negative	22	15 (68.2)	24 (22-26)		48 (23-73)	
PR				0.733		0.012
Positive	8	2 (25.0)	26 (5-47)		129 (96-162)	
Negative	26	19 (73.1)	25 (21-29)		56 (33-79)	
AR				0.020		0.015
Positive	8	2 (25.0)	60 (13-107)		193 (133-253)	
Negative	26	19 (73.1)	24 (22-26)		58 (32-84)	
HER-2				0.583		0.261
Positive	8	5 (62.5)	25 (13-37)		38 (1-75)	
Negative	26	16 (61.5)	25 (20-30)		70 (43-97)	
CK5				0.109		0.616
Positive	6	4 (66.7)	14 (1-27)		36 (4-68)	
Negative	28	17 (60.7)	27 (14-40)		70 (50-90)	
p53				0.472		0.171
Positive	5	4 (80.0)	25 (19-31)		38 (34-42)	
Negative	29	17 (58.6)	26 (21-31)		70 (46-94)	
E-cadherin				0.589		0.461
3	6	2 (33.3)	24 (22-26)		79 (47-111)	
2	21	13 (61.9)	27 (8-46)		60 (34-86)	
1	3	2 (66.7)	33 (15-51)		56 (36-64)	
0	4	4 (100.0)	24 (10-38)		36 (0-77)	
Bcl-2				0.031		0.036
Positive	13	6 (46.2)	38 (12-64)		153 (100-206)	
Negative	21	15 (71.4)	24 (22-26)		48 (24-72)	
Vimentin				0.191		0.984
Positive	9	5 (55.6)	24 (18-30)		70 (32-108)	
Negative	25	16 (64.0)	27 (14-40)		58 (29-87)	
EGFR				0.073		0.392
3	2	1 (50.0)	11 (n/a)		94 (41-147)	
2	2	1 (50.0)	13 (10-15)		36 (n/a)	
1	4	3 (75.0)	22 (2-42)		36 (6-66)	
0	26	16 (61.5)	27 (15-39)		70 (47-93)	
VEGF				0.755		0.609
3	4	3 (75.0)	23 (22-24)		48 (34-62)	
2	10	7 (70.0)	25 (20-30)		60 (7-113)	
1	10	6 (60.0)	33 (14-52)		56 (0-137)	
0	10	5 (50.0)	24 (2-46)		72 (66-78)	
c-kit				0.658		0.866
Positive	4	2 (50.0)	14 (0-43)		52 (30-73)	
Negative	30	19 (63.3)	25 (21-29)		60 (43-77)	
IHC subtype				0.158		0.120
ER-/PR-/HER-2-	13	8 (61.5)	23 (12-34)		48 (30-66)	
HER-2+	8	5 (62.5)	25 (13-37)		38 (1-75)	
ER or PR+/HER-2-	13	8 (61.5)	38 (10-66)		108 (95-191)	
Organ				0.053		0.000
Lung	15	5 (33.3)	35 (7-63)		171 (120-221)	
Liver	4	3 (75.0)	23 (0-57)		60 (15-105)	
Bone	9	8 (88.9)	24 (21-27)		58 (29-87)	
Brain	6	5 (83.3)	15 (1-29)		24 (21-27)	

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; CK5, cytokeratin 5; EGFR, epithelial growth factor receptor; VEGF, vascular endothelial growth factor; IHC, immunohistochemistry; n/a, not available.

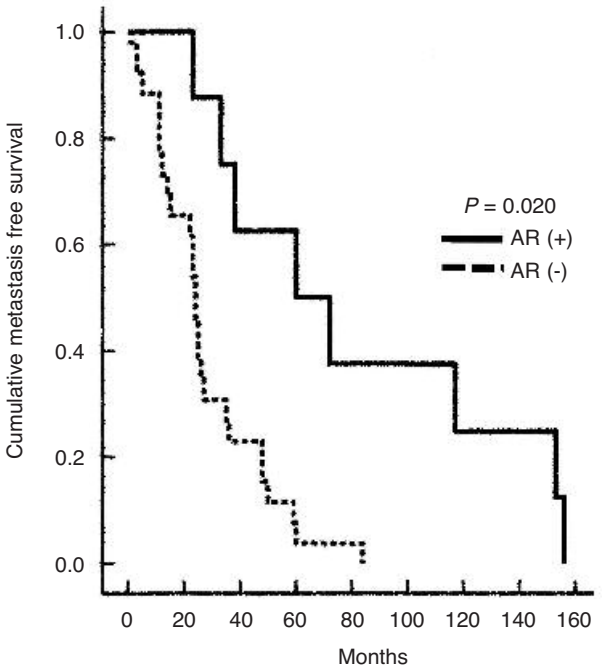

Figure 1 - Comparison of metastasis-free survival curve between metastatic breast cancer with androgen receptor (AR) expression and metastatic breast cancer without AR expression.

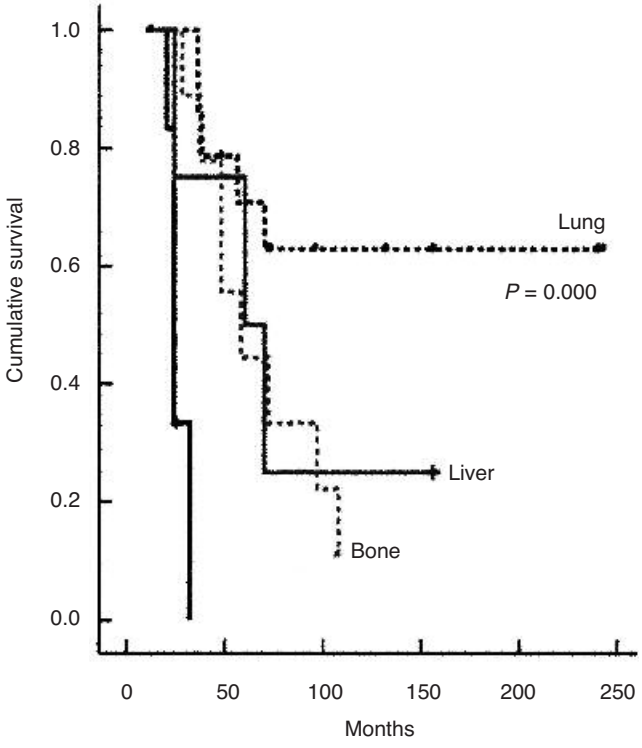

Figure 2 - Comparison of overall survival curve among organs where tumor metastasis occurred.

sion loss of c-kit was observed in the process of breast cancer progression. Therefore, it has been suggested that c-kit may play a role in breast cancer progression[26]. C-kit expression in metastatic breast cancer was reported to be from 0 to 3%[26,27]. Further study on whether c-kit could be a useful marker of targeted therapy in patients with metastatic breast cancer showing gain of c-kit expression should be performed.

In the analysis of 34 cases of metastatic breast cancer, the disease showed a different immunohistochemical phenotype according to metastatic organ (P = 0.048). Metastatic breast cancer in the brain showed mainly the HER-2+ type (66.7%), and metastatic breast cancer in the liver revealed mainly the ER+ or PR+/HER-2- type (75.0%). The ER+ or PR+/HER-2- type (55.6%) and triple negative type (44.4%) were seen in metastatic breast cancer in the bone, and metastatic breast cancer in the lung showed all three subtypes in similar proportions. Studies of metastatic breast cancer have extensively focused on brain and bone metastasis. Associated factors for brain metastasis in breast cancer are young age, ER negativity, prior lung metastasis, HER-2 overexpression, EGFR overexpression, and basal subtype[9-11]. In the present study, brain metastasis demonstrated ER, PR, and AR negativity and showed HER-2 overexpression but EGFR or CK5 expression was not noted. The exact mechanism by which breast cancer with HER-2 overexpression gives rise to brain metastasis is not fully known, but a possible explanation is that HER-2 overexpression at first gives tumor cells intrinsic metastatic aggressiveness, causing brain metastasis[28]. In addition, with trastuzumab therapy prolonging patient survival, the possibility of brain metastasis is increased[11]. Finally, because trastuzumab does not penetrate the blood-brain barrier, an effective treatment for brain metastasis cannot be performed[1].

The reported associated factors in bone metastasis are low histologic grade, ER positivity, ER positivity/PR negativity, lymph node staging, strand growth pattern, and the presence of fibrotic foci in invasive ductal carcinoma[8,29,30]. The present study revealed that bone metastasis was composed of the ER or PR+/HER-2- subtype (55.6%) and triple-negative subtype (44.4%). The ER or PR+/HER-2- subtype is generally known to be of low grade but the triple-negative subtype is known to be high grade. Therefore, bone metastasis showed both low- and high-grade breast cancers.

The characteristics of breast cancer that give rise to lung metastasis are tumor necrosis, ER and PR negativity, skin invasion, nodal stage (N) 3, and lymph vessel invasion[29]. In addition, other studies have reported that N3, vascular invasion, and ER/PR/HER-2/p53 negativity were associated with lung metastasis[31]. Although lung metastasis in our study showed a tendency of HER-2 and p53 negativity, the triple-negative subtype (33.3%) as well as the ER or PR+/HER-2- subtype (40.0%) were observed in lung metastasis. Liver metastasis is report-

ed to be associated with N3, ER and PR negativity, and fibrotic focus in invasive ductal carcinoma[29]. However, 75% of liver metastasis in this study was the ER or PR+/HER-2- subtype. Most previous studies investigated and used tissues of only primary breast cancer to identify the factors associated with distant metastasis[1,8,9,11,29]. However, there have been a few studies that used tissues of metastatic breast cancer to investigate the characteristics of metastatic breast cancer, as in this study[10,12]. Therefore, there may be a discrepancy between the characteristics of primary breast cancer, which gives rise to distant metastasis, and those of metastatic breast cancer. Furthermore, as observed in the 13 cases of paired primary and metastatic breast cancer, there can be a shift in expression of biological factors such as ER and PR between primary and metastatic breast cancer.

In the present study, among clinopathologic and immunohistochemical parameters, ER ($P = 0.037$), AR ($P = 0.020$) and Bcl-2 ($P = 0.031$) showed statistical significance in time to metastasis-free survival. Nuclear grade ($P = 0.005$), PR ($P = 0.012$), AR ($P = 0.015$), Bcl-2 ($P = 0.036$), and metastatic organ ($P = 0.000$) demonstrated statistical significance in time to overall survival. Of these parameters, especially AR displayed significance in time to metastasis-free survival and overall survival. In addition, only AR ($P = 0.001$) showed statistical significance in multivariate analysis of time to metastasis-free survival. The previous studies reported that AR was a prognostic factor along with ER, tumor size, tumor grade, lymph node status, and high level of Ki-67[32]. In breast cancer with ER expression, AR-expressing breast cancer exhibited significantly better disease-free survival than breast cancer without AR expression[33]. In addition, AR-positive invasive ductal carcinomas have been associated with a low or intermediate histological grade[34-36]. These results indicate that breast cancer with AR expression shows a better prognosis than breast cancer without AR expression, which is compatible with the results of this study showing that metastatic breast cancer with AR expression showed longer metastasis-free survival and overall survival than metastatic breast cancer without AR expression.

Most patients with metastatic breast cancer are managed by medical rather than surgical treatment, making it is very difficult to obtain tissues from metastatic tumors. We could not investigate a sufficient number of metastatic tumor tissues, which is a major limitation of this study.

In conclusion, metastatic breast cancer shows different immunohistochemical phenotypes according to metastatic site ($P = 0.048$). HER-2+ type (66.7%) in brain metastasis and ER+ or PR+/HER-2- type (75.0%) in liver metastasis were predominant. Bone metastasis was composed of the triple-negative type (44.4%) and ER+ or PR+/HER-2- type (55.6%), and lung metastasis showed all three subtypes in similar proportions.

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