Comparative Study of Metaplastic Breast Carcinoma and Triple-Negative Breast Carcinoma Using Histologic and Immunohistochemical Analyses

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Background : Metaplastic carcinoma of the breast is a rare subtype of breast cancer, which is characterized by estrogen receptor/progesterone receptor and HER2 negativity. **Methods :** Tissue specimens from 60 metaplastic breast cancer and 60 triple-negative breast cancer patients diagnosed at a single institution between 1995 and 2009 were analyzed. Immunohistochemistry for caveolin-1 (CAV-1), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), c-kit, p53, Ki-67, breast cancer type 1 susceptibility protein (BRCA1), cytokeratin (CK)14, and CK17 were performed on both retained tissue sets. **Results :** Of the 60 metaplastic carcinomas, 15 tumors (25%) exhibited spindle cell component, 27 (45%) exhibited chondroid differentiation, and 18 (30%) exhibited squamous areas. Compared to triple-negative carcinomas, metaplastic carcinomas significantly more frequently expressed CK14 (p < 0.0001), CK17 (p = 0.002), EGFR (p < 0.0001), CAV-1 (p < 0.0001), and VEGF (p = 0.029). However, expressions of BRCA1, p53, c-kit, and Ki-67 were not significantly different between both groups. **Conclusions :** The expression profile of metaplastic carcinoma of the breast is more homogeneous than that of other triple-negative tumors and frequently over-expresses basal markers, CAV-1, and VEGF. A typical "basal-like" phenotype and frequent expressions of CAV-1 and VEGF may justify specific therapeutic approaches.

Key Words : Metaplastic carcinoma; Breast; Triple-negative cancer; Immunohistochemistry

Breast cancer is managed with a combination of surgery, medical therapy, and radiotherapy. Of these therapeutic options, medical therapy such as hormonal therapy, chemotherapy, and targeted therapy show variable responses and are dependent upon tumor histologic subtype and biomarker expression. The optimal management of breast cancer patients requires a tumor-specific and a patient-specific approach. For example, trastuzumab is available as an adjuvant therapy for tumors that over-express human epidermal growth factor receptor 2 (HER-2) protein, or show amplification of the *HER2/neu* gene.[1,2] Endocrine therapy works best in women whose tumors are positive for esrtrogen receptor (ER) and/or progesterone receptor (PR). Therefore, data concerning tumor type aid therapeutic decision-making.

Metaplastic carcinoma of the breast is a heterogeneous group of uncommon malignant tumors, comprised of glandular and non-glandular components. The latter of which may be spindle, squamous, or chondroid.[3] Metaplastic carcinomas are almost invariably negative for ER, PR, and HER-2. As a consequence,

adjuvant treatment is limited in patients with metaplastic carcinoma, and in this regard, metaplastic carcinoma is similar to other triple-negative breast cancers. At the same time, metaplastic carcinoma of the breast is considered a distinct tumor subtype with specific characteristics that differentiate it from more common malignant breast histologies.[4,5] However, few studies have attempted to delineate factors that distinguish metaplastic carcinoma from other triple-negative breast cancers. We hypothesized metaplastic carcinoma is a single entity with variable morphologic features, but more homogeneous than other triple-negative carcinomas, and more specifically, it has more homogeneous immunohistochemical characteristics than other triple-negative breast cancers. If the above hypotheses were proven right, they could help identify novel therapeutic targets to manage metaplastic carcinomas.

In this study, we compared metaplastic breast carcinoma and triple-negative breast carcinoma with respect to the immuno-expressions of caveolin-1 (CAV-1), vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), c-kit,

p53, Ki-67, breast cancer type 1 susceptibility protein (BRCA1), cytokeratin (CK)14 and CK17. In addition, we attempted to seek clinicopathological correlations.

MATERIALS AND METHODS

Patients

Histopathologic data files at the Samsung Medical Center were reviewed to identify metaplastic breast carcinoma. Formalin-fixed, paraffin-embedded blocks of metaplastic breast cancer specimens were identified from 60 patients who underwent surgical resection at the center between January 1995 and June 2009. Hematoxylin and eosin-stained slides of tumors were reviewed for tumor type confirmation in all cases. Clinicopathological parameters, such as age, tumor size, tumor grade, lymph node status, distant metastasis, and hormone receptor status were obtained by reviewing medical charts and pathological records.

Sixty (n = 60) surgically resected, triple-negative breast carcinoma specimens were included for the purpose of comparison. Clinicopathologic data, including hormone receptor status were obtained by reviewing medical charts and pathological records. All 60 triple-negative cases were negative for ER and PR and did not overexpress HER-2.

Assembly of the tissue microarray

Surgical specimens were fixed in 10% buffered formalin, processed, and embedded in paraffin using a standard protocol. Representative areas on hematoxylin and eosin-stained sections were carefully selected and marked on individual paraffin blocks. Two tissue cores (2-mm diameter) were obtained from each case. These tissue cores were arrayed in recipient paraffin blocks according to the manufacturer's instructions.

Immunohistochemistry using tissue array blocks

After deparaffinization and rehydration, $4\,\mu$m-thick sections on saline-coated slides were heat-pretreated with citrate buffer (pH 7.3 at 92℃ in a microwave oven), and examined by immunostaining using specific antibodies against CAV-1 (BD Biosciences, San Jose, CA, USA), VEGF (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), EGFR (Novocastra, Newcastle, UK), c-kit (Dako, Glostrup, Denmark), p53 (Novocastra), Ki-67 (Dako), BRCA1 (Abcam, Cambridge, UK),

CK14 (Dako), and CK17 (Dako). The avidin-biotin technique with DAB was used for visualization, and hematoxylin for nuclear counterstaining.

Interpretation of immunohistochemical staining results

The tissue array blocks initially contained 63 metaplastic carcinomas and 65 triple-negative carcinomas, respectively. However, 3 metaplastic carcinomas and 5 triple-negative carcinomas were excluded due to tissue loss, and thus, 60 metaplastic carcinomas and 60 triple-negative carcinomas were analyzed in this study. All immunostain was evaluated by two pathologists blinded to clinical and pathological data. Generally, their findings correlated well. When their findings differed, final interpretations were reached by consensus. For ER, PR, and HER-2, interpretations of immunostaining were made on the overall metaplastic component and invasive ductal carcinoma component, because we used slides that had been immunostained in routine diagnosis. For other markers, interpretations of immunostaining were made on the most predominant metaplastic component, because immunostains were performed on array blocks.

ER, PR, p53, BRCA1, and Ki-67 expression were located in the nuclei, HER-2 in the membranes, VEGF, CK14 and CK17 in the cytoplasm, and c-kit, CAV-1, and EGFR in the cytoplasm and/or membranes. ER and PR immunoreactivity was scored by evaluating staining intensities (0-3), and the proportions (0-5) of tumor nuclei stained using the Allred scoring system. The sums of these scores are referred to as ER or PR scores. A score from 0 to 2 was conferred hormone receptor negative status.[6]

HER-2 immunoreactivity was assessed using the following scoring approach: 0, no immunoreactivity or immunoreactivity in < 10% of tumor cells; 1+, faint weak and incomplete staining of > 10% of tumor cells; 2+, weak to moderate complete membrane immunoreactivity in > 10% of tumor cells; and 3+, moderate to strong complete membrane immunoreactivity in > 10% of tumor cells. Tumors with intensities of 0 or 1+ were considered negative.

p53 staining was defined as positive if the percentage of cells with antibody staining was ≥ 5%.

Ki-67 was scored according to the percentage of tumor nuclei labeled by Ki-67, the scores of which ranged from 1+ to 4+, where 1+, < 25% positive cells; 2+, ≥ 25% but < 50%; 3+, ≥ 50% but < 75%; and 4+, ≥ 75%.

Immunohistochemical stainings for CAV-1, VEGF, EGFR,

c-kit, BRCA1, CK14, and CK17 were scored based on intensity (0-3+) and percentage (0-100%). The percentage of positive tumor cells and the staining intensity were then multiplied, in order to generate the immunoreactivity score for each tumor specimen. The results were considered to be positive when the score was ≥ 10.

Statistical analysis

Comparative analysis of immunohistochemical results was conducted using Fisher's exact test, Pearson's chi-square test, ANOVA, Mann-Whitney tests, and Kruskal-Wallis test. Statistical significance was reached when p-value was < 0.05. All analyses were performed using the PASW ver. 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinicopathological findings of metaplastic carcinoma and triple-negative carcinoma

The age range of the 60 metaplastic carcinoma patients was 25-83 years, and the mean patient age was 47.6 years. All patients underwent curative resection. Twenty-one patients underwent partial mastectomy with sentinel node biopsy, 20 underwent partial mastectomy with axillary dissection, and 19 underwent modified radical mastectomy. The follow-up periods ranged from 1 to 134 months (average, 38 months). Tumor sizes were between 5 and 60 mm (mean, 27 mm). Nineteen (31.7%) patients experienced lymph node metastasis. In 19 patients with lymph node metastasis, the mean number of positive nodes was 2.4 (range, 1 to 11). Fourteen patients presented with American Joint Committee on Cancer (AJCC, 7th edition) stage IA, and 29 with stage IIA disease. Tumor stage at time of diagnosis was IIB and IIIA in 12 and 4 patients, respectively. One patient was classified as stage IIIC. Modified Bloom-Richardson grade and nuclear grade were available in all cases; 46 (76.7%) were of histologic grade III (8-9), 10 (16.7%) were of grade II (6-7), and 4 (6.7%) were of grade I (3-5). Nuclear grade was high in 48 (80.0%), intermediate in 11 (18.3%), and low in 1 (1.7%). Eight patients experienced distant metastases at a mean of 21.3 months postoperatively (range, 9 to 56 months). No local recurrences were identified. Common metastatic sites were brain, lung and bone. Of the 8 patients with metastasis, 5 died of the disease at a mean of 32.2 months postoperatively (range, 15 to 65 months), and at a mean of 6.2 months from the onset of metastasis (range, 1 to 13 months).

The triple-negative group comprised of patients with advanced cancers, compared to the metaplastic group, with all patients having experienced progression to metastatic carcinoma. Among 60 patients with triple-negative carcinomas, 4 patients underwent partial mastectomy with sentinel node biopsy, 17 underwent partial mastectomy with axillary dissection and 39 underwent modified radical mastectomy. The mean age of the triple-negative group was 44.9 years, which ranged from 22 to 72 years. The follow-up periods ranged from 4 to 150 months, with a mean of 43 months. Tumor sizes ranged from 6 to 80 mm with a mean of 30 mm. Forty-one (68.3%) patients in the triple-negative group experienced lymph node metastasis, with a mean of 9.5 metastatic nodes. Tumor stages at the time of diagnosis were IA in 8 triple-negative cancer patients, IIA in 13, IIB in 13, IIIA in 9 and IIIC in 16 patients. The Modified Bloom-Richardson grade of 12 patients was II, and that of other 48 patients was III. Fourteen cases showed intermediate nuclear grade and the remaining 46 cases showed high nuclear grade. As previously mentioned, all patients included in this triple-negative group experienced distant metastasis, and the mean postoperative time to the metastasis was 24.9 months. Frequent metastatic sites were lung, bone, brain and liver. Thirty-eight patients in this group died, with a mean follow-up period of 38.0 months.

Table 1 summarizes the clinical and pathologic characteristics of metaplastic carcinomas and triple-negative carcinomas.

Table 1. Tumor characteristics

	Metaplastic carcinoma	Triple-negative carcinoma
No. of cases	60	60
Mean age (yr)	47.6	44.9
Tumor size (cm)		
< 2	16 (26.7)	15 (25.0)
2-5	41 (68.3)	38 (63.3)
> 5	3 (5.0)	7 (11.7)
Nuclear grade		
1	1 (1.7)	0
2	11 (18.3)	14 (23.3)
3	48 (80.0)	46 (76.7)
Histologic grade		
I	4 (6.7)	0
II	10 (16.7)	12 (20.0)
III	46 (76.7)	48 (80.0)

Values are presented as number (%).

Microscopic features of metaplastic carcinoma and triple-negative carcinoma

Microscopically, each metaplastic carcinoma showed invasive ductal carcinoma with foci of squamous carcinomatous, chondromyxoid, and spindle cell components in the tumor. When cases were categorized according to predominant components, 15 cases (25%) were of spindle cell subtype, 27 (45%) were of the matrix-producing subtype, and 18 (30%) were squamous cell carcinomas. Ten cases had more than one metaplastic component; spindle-squamous-matrix (n = 1), spindle-squamous (n = 2), spindle-matrix (n = 5), spindle-osteoclastic giant cells (n = 1), and matrix-squamous (n = 1). One patient had a heterologous component with an osteosarcoma-like area. Forty-three patients (71.7%) showed tumor necrosis and 6 (10%) showed a dense peritumoral inflammatory reaction. Extensive intraductal components were identified in 6 patients (10%). Disease free survival (DFS) for each subtype was 86.7% for the spindle cell subtype, 94.4% for the squamous subtype and 92.6% for the matrix-producing subtype. DFS in all categories showed no significant association with histologic subtypes (Table 2).

The triple-negative group included 1 secretory carcinoma, 1 invasive micropapillary carcinoma, 1 invasive cribriform carcinoma and 2 medullary carcinoma cases. The remaining 55 cases were of invasive ductal carcinomas, not otherwise specified. Of these 60 cases, 39 cases showed tumor necrosis and 6 cases showed peritumoral inflammatory reaction. Eight cases demonstrated extensive intraductal components. The microscopic features of the triple-negative group, including inflammation, necrosis and intraductal component, were similar to that of metaplastic group.

Biomarker expression

Biomarker expressions were examined in the metaplastic carcinoma group and in the triple-negative carcinoma group.

Fifty-seven out of 60 metaplastic carcinomas were negative for ER, and 55 were negative for PR. Only 1 patient showed +2 staining for HER-2, but fluorescence in situ hybridization for HER2/neu gene amplification was not performed in this patient. All triple-negative carcinomas were negative for ER, PR, and HER-2 by definition.

When compared to triple-negative carcinomas, metaplastic carcinomas significantly more frequently expressed basal markers, such as CK14 (37 vs 16, $p < 0.0001$), CK17 (35 vs 18, $p = 0.003$), and EGFR (56 vs 39, $p < 0.0001$). The number of cases expressing at least one basal marker was also significantly more frequent in the metaplastic carcinoma group (59 vs 51, $p = 0.008$). Furthermore, the frequencies of CAV-1(41 vs 19, $p < 0.0001$) and VEGF (45 vs 33, $p = 0.022$) expression were also significantly higher in the metaplastic carcinoma group (Fig. 1). Frequencies of c-kit expressions were similar in the two groups (32 vs 29, $p = 0.584$), as were those of BRCA1 (53 vs 49, $p = 0.306$) and p53 (43 vs 39, $p = 0.432$). Ki-67 labeling indices were similar between the two groups (Table 3).

No significant differences were observed for the different subtypes of metaplastic carcinoma in terms of immunohistochemical results (Table 4).

Table 3. Immunohistochemical results of metaplastic carcinoma and triple-negative carcinoma

Antibodies	Metaplastic carcinoma	Triple-negative carcinoma	p-value
BRCA1	53 (88.3)	49 (81.7)	NS
p53	43 (71.7)	39 (65.0)	NS
EGFR	56 (93.3)	39 (65.0)	0.000
CK14	37 (61.7)	16 (26.7)	0.000
CK17	35 (58.3)	18 (30.0)	0.003
c-kit	32 (53.3)	29 (48.3)	NS
Basal markers	59 (98.3)	51 (85.0)	0.008
VEGF	45 (75.0)	33 (55.0)	0.022
CAV-1	41 (68.3)	19 (31.7)	0.000

Values are presented as number (%). Basal markers: cases expressing at least one basal marker including EGFR, CK14, CK17 and c-kit. BRCA1, breast cancer type 1 susceptibility protein; NS, not significant; EGFR, epidermal growth factor receptor; CK, cytokeratin; VEGF, vascular endothelial growth factor; CAV-1, caveolin-1.

Table 2. Microscopic features of metaplastic carcinoma

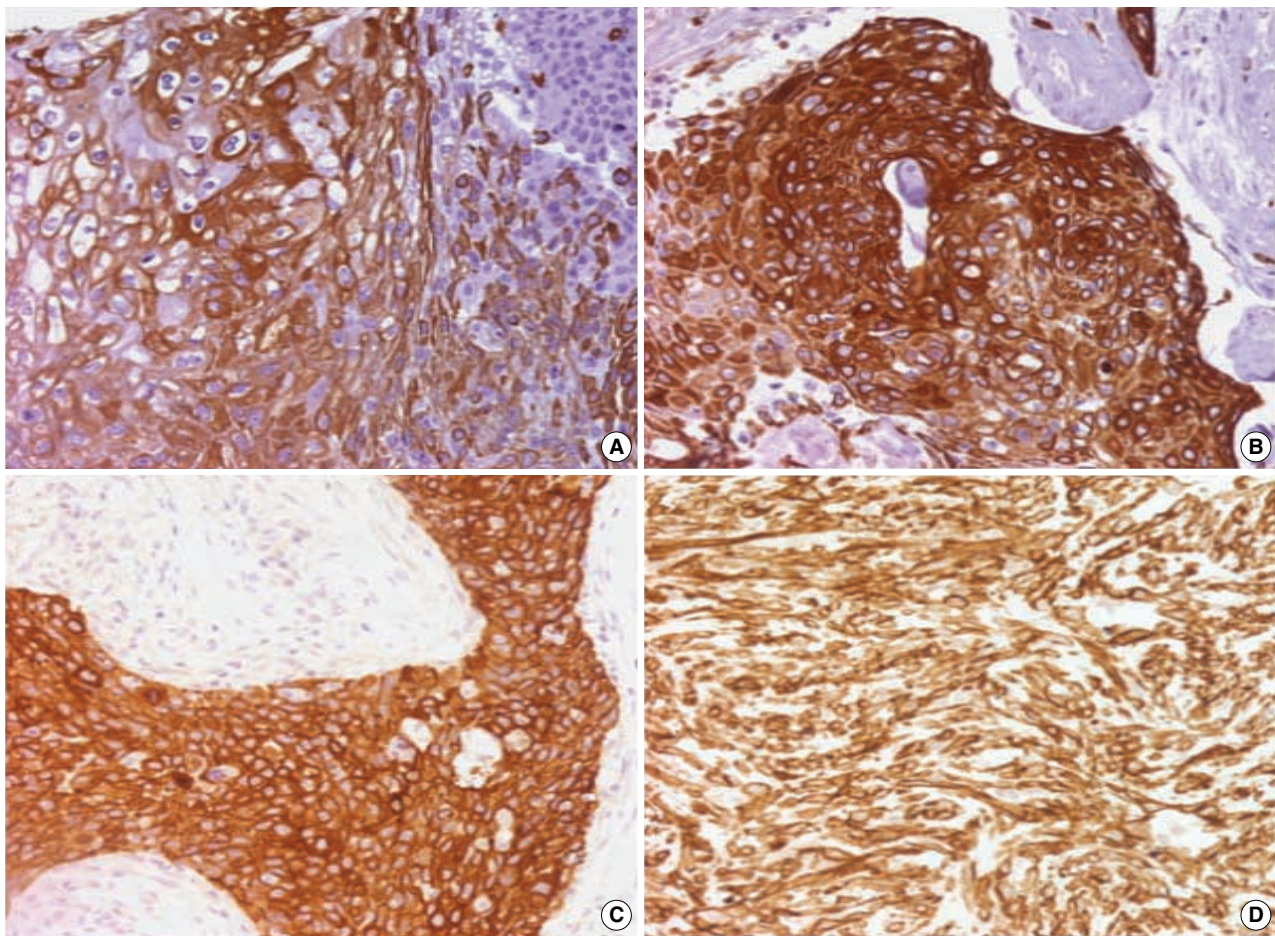| Histologic subtypes	Mean age (yr)	Tumor size (cm)	Nodal status No. (%)	DFS
Adenocarcinoma with				
Spindle cell type (n = 15)	47.7 (31-65)	3.5 (0.5-6.0)	5 (33.3)	86.7%
Squamous type (n = 18)	47.0 (27-70)	2.5 (1.0-5.5)	6 (33.3)	94.4%
Matrix-producing type (n = 27)	48.0 (25-83)	2.4 (0.8-4.2)	8 (29.6)	92.6%
Overall	47.6 (25-83)	2.7 (0.5-6.0)	19 (31.7)	91.7%

DFS, disease free survival.

Fig. 1. Immunohistochemical findings for biomarkers in metaplastic carcinoma. (A) Immunostaining result for cytokeratin (CK)14. (B) Immunostaining for CK17. (C) Immunostaining for epidermal growth factor receptor. (D) Immunostaining for caveolin-1.

Table 4. Immunohistochemical results of metaplastic carcinoma with respect to predominant metaplastic components

Antibodies	Spindle type (n = 15)	Squamous type (n = 18)	Matrix-producing (n = 27)	p-value
BRCA1	13 (86.7)	17 (94.4)	23 (85.2)	NS
p53	12 (80.0)	12 (66.7)	19 (70.4)	NS
EGFR	14 (93.3)	16 (88.9)	26 (96.3)	NS
CK14	9 (60.0)	10 (55.6)	18 (66.7)	NS
CK17	9 (60.0)	11 (61.1)	15 (55.6)	NS
VEGF	13 (86.7)	14 (77.8)	18 (66.7)	NS
CAV-1	9 (60.0)	12 (66.7)	20 (74.1)	NS
c-kit	7 (46.7)	5 (27.8)	20 (74.1)	NS

Values are presented as number (%).
BRCA1, breast cancer type 1 susceptibility protein; NS, not significant; EGFR, epidermal growth factor receptor; CK, cytokeratin; VEGF, vascular endothelial growth factor; CAV-1, caveolin-1.

DISCUSSION

Metaplastic carcinoma is a heterogeneous group of breast cancers characterized by various components, including adenocarcinomatous, other epithelial, and mesenchymal compo-nents. According to the components present, Wargotz *et al.* described five subgroups of metaplastic carcinoma, namely, matrix-producing carcinoma, spindle cell carcinoma, carcinosar-coma, squamous cell carcinoma of ductal origin, and metaplas-tic carcinoma with osteoclastic giant cells.[7-11] In the present

study, 10 metaplastic carcinoma cases exhibited an admixture of more than one metaplastic component, although the volume of the minor component was very small. Osako *et al.*[12] reported a case with ductal, squamous, and sarcomatous components. In the present study, we found no significant difference in the prognoses of these subtypes, which concurs with the findings of a previous study.[13] In addition, in the present study, biomarker expressions were similar between different subtypes.

The present study is limited in terms of its ability to identify relationships between immunohistochemical staining status and survival, because of the relatively small number of cases included. However, DFS in our metaplastic carcinoma patients was high (at 91.7%) and did not differ by subtype, and this finding does not concur with previous reports.[4,5,14,15] Our relatively high DFS may be associated with the fact that our metaplastic carcinoma group comprised of early carcinomas with low positive lymph node rates. In addition, the short follow-up period may be a contributing factor. Another limitation in our study was that it was impossible to compare the survivals of the metaplastic carcinoma group with the triple-negative group, because the two cohorts were not stage-matched and too small to enable evaluation of survival. In contrast to the metaplastic carcinoma group, the triple-negative carcinoma group comprised of advanced carcinomas progressing to metastasis in all cases. The advanced stage of triple-negative group is responsible for the reduced survival, which rendered comparison of survival between the two groups not feasible.

Basal-like breast carcinoma is a term used to describe a biologically diverse group of breast cancers with different clinical features and outcome, including medullary carcinoma and adenoid cystic carcinoma.[16] Basal-like carcinomas are defined by gene expression profiling. Gene expression profiling-based molecular classification categorized breast cancer into luminal A, luminal B, normal breast, HER2+ and basal-like subtypes.[17-19] Of these, basal-like subtype is characterized by expression of basal markers, such as CK14, CK17, CK5/6, EGFR, c-kit, and frequently associated with BRCA1.[20] Among these markers, we performed immunostainings for CK14, CK17, EGFR, c-kit, BRCA1. The results suggest that metaplastic carcinomas more frequently expressed CK14 (37 vs 16, p < 0.0001), CK17 (35 vs 18, p = 0.003), and EGFR (56 vs 39, p < 0.0001) compared to triple-negative carcinomas. In view of the fact that CK14, CK17 and EGFR are basal markers, the present study shows that metaplastic carcinomas more frequently express basal markers than other triple-negative carci-

nomas. Previous studies showed that metaplastic breast carcinomas are basal-like cancers based on typical immunoprofile of basal-like tumors[21] and a genomic profiling analysis,[22] and the result of this study was concordant with that.

Metaplastic carcinomas are almost invariably negative for ER, PR, and HER-2. As a consequence, adjuvant treatment is limited in patients with metaplastic carcinoma, as is the case in other triple-negative breast cancers. Although the present study involved a relatively small number of cases, our results demonstrate that metaplastic breast carcinoma frequently overexpresses EGFR, VEGF and CAV-1, which can be used as therapeutic targets. Leibl and Moinfar[23] showed that 14 of the 20 metaplastic carcinomas (70%) were positive for EGFR immunostaining, and Savage *et al.*[24] showed that CAV-1 was expressed in 90% of 39 metaplastic breast carcinomas and in 9.4% of 245 invasive breast cancers. In our study, 56 out of 60 metaplastic carcinomas (93.3%) expressed EGFR, and 41 out of 60 (68.3%) expressed CAV-1. These results are not different from that obtained in previous studies. However, in contrast to a previous study, we used the triple-negative carcinoma group as a control cohort, and compared the immunoprofiles between the two groups. It must be emphasized that the frequency of positive VEGF immunostaining in metaplastic breast carcinoma has not been reported previously.

Recently, molecular-targeting agents against the above molecules have attracted the attention of breast cancer patients. Target-directed therapies with monoclonal antibodies and small-molecule inhibitors have improved the therapeutic outcomes of cancer patients when combined with cytotoxic agents or radiation therapy. Anti-EGFR antibodies that specifically prevent aberrant intracellular signaling activities for tumor cell survival and proliferation are effective monotherapies in patients with advanced, chemotherapy-refractory cancer. These agents are able to potentiate the antitumor efficacy of chemotherapy in cancer patients.[25] A phase II study is currently underway to evaluate the effect of Erlotinib in triple negative carcinoma. Furthermore, a VEGF-A neutralizing antibody that blocks VEGF-A signaling for tumor angiogenesis has been approved by the US Food and Drugs Administration (FDA) as treatment for metastatic breast cancer in combination with chemotherapy, which supports the notion that the VEGF/VEGF receptor (VEGFR) signaling pathways are promising targets for cancer intervention.[26] In addition, a phase II study is ongoing to investigate chemotherapy vs Sunitinib malate, which targets several moieties including VEGFR1, VEGFR2, platelet-derived growth factor receptor and KIT, in anthracycline and in tax-

ane pretreated patients.[27] CAV-1 is believed to have an important impact on both signal transduction and on the mediation of intracellular processes.[28] Recent results have revealed that CAV-1 has potential therapeutic relevance. Bortezomib, an antibody against 26-S-proteasome, has been shown to target CAV-1 among a variety of proteins, and a preclinical study demonstrated that Bortezomib has antitumor activity.[29,30] Although the roles of differentially expressed biomarkers are not clear, our finding that metaplastic breast carcinoma differentially expresses EGFR, VEGF and CAV-1 more frequently than other triple-negative carcinomas support the contention that metaplastic breast carcinomas may respond better to therapies targeting EGFR, VEGF and CAV-1.

In conclusion, our results suggest that the expression profile of metaplastic carcinoma of the breast tends to be more homogeneous than that of other triple negative carcinomas, despite its morphologic heterogeneity. Although it is not clear whether metaplastic carcinoma is more predictable in terms of its response to molecular targeting therapy than other triple negative carcinomas, overexpression of basal markers, CAV-1 and VEGF at the protein level may justify specific therapeutic approaches.

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