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Clinicopathological Significance of Invasive Ductal Carcinoma with High Prevalence of $CD44^{+}/CD24^{-/low}$ Tumor Cells in Breast Cancer

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Background : Epithelial tumor cells with a $CD44^{+}/CD24^{-/low}$ immunoprofile may have the ability to cause breast cancer. We studied these cells and their clinicopathological significance. **Methods :** The clinicopathologic findings of 100 invasive ductal carcinoma (IDC) cases and 45 ductal carcinoma *in situ* (DCIS) cases were reviewed. $CD44^{+}/CD24^{-/low}$ tumor cells were identified by immunohistochemistry, and their clinicopathological implications in IDC and DCIS were analyzed. **Results :** IDC with a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells was significantly associated with larger mass, higher grade, estrogen receptor (ER) negativity, and tumor cells with a higher frequency of metastasis. The proportion of $CD44^{+}/CD24^{-/low}$ tumor cells in IDC, and its DCIS components was not significantly different, whereas the proportion of $CD44^{+}/CD24^{-/low}$ tumor cells was higher in DCIS than in the DCIS component of IDC ($p < 0.001$). **Conclusions :** IDC with a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells might correlate with aggressive features, such as ER and higher grades. Moreover, the proportion of $CD44^{+}/CD24^{-/low}$ tumor cells in the DCIS components of IDC and DCIS might harbor different biology, which may lead to differences in cancer progression and early carcinogenesis.

Key Words : Neoplastic stem cells; CD44 protein, human; CD24 protein, human; Invasive ductal carcinoma; Carcinoma, intraductal, noninfiltrating

Breast cancer is the most common malignancy in females and the leading cause of cancer-related death in women worldwide.[1] Breast cancer is a highly heterogeneous disease, which displays varying molecular and clinical features, and therapeutic resistance is a frequently encountered problem. The cancer stem cell (CSC) hypothesis was proposed to explore the cause of breast cancer's diverse genetics and pathological patterns. According to this hypothesis, only rare CSCs have indefinite proliferative potential and drug resistance that drives tumor progression and recurrence.[2] Although non-CSCs actively proliferate and constitute the majority of tumor volume, they are differentiated and destined to die. This hypothesis is very attractive, because a therapeutic strategy directed at CSCs would be very useful.

Since $CD34^{+}/CD38^{-}/CD90^{-}$ cells were identified as CSCs in acute myelogenous leukemia, CSCs identified in several solid tumors have also demonstrated an ability to initiate tumor growth in immunocompromised mice.[3,4] For example, $CD44^{+}/CD24^{-/low}$ cells existing as breast cancer stem cells (BCSCs) cause breast cancer in immunocompromised mice.[5]

The clonal evolution model suggests that tumor cell phenotypes are determined by a combination of an originating cell type of the tumor-initiating cells, acquired genetic and epigenetic alterations, and microenvironmental effects. Thus, in the clonal evolution model, all tumor cells show unstable phenotypes and have the capacity for self-renewal, progression, and drug resistance. Despite a plethora of breast cancer studies, no consensus exists concerning the heterogeneity of breast cancer.

In this study, we investigated the prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells in human breast cancer and analyzed the clinicopathological significance and prognostic value of this cell type.

MATERIALS AND METHODS

Patients

One hundred patients with breast cancer who were diagnosed with invasive ductal carcinoma (IDC) and 45 breast can-

cer patients who were diagnosed with ductal carcinoma *in situ* (DCIS) at Kyung Hee University Medical Center from 2000 to 2007 were recruited into the study. Every case was diagnosed from surgically-resected specimens, and clinical data were collected by retrospective review. Results of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2/Neu) status were also obtained from medical records. ER and PR immunoreactivity was evaluated according to the Allred scoring method.[6] For Her2/Neu immunoreactivity, only membranous staining was scored according to criteria defined in the HerceptTest (DAKO, Carpinteria, CA, USA) protocol.[7] Her2/Neu-positive was defined as 3+ on immunohistochemistry (IHC) or 2+ and fluorescence positivity on *in situ* hybridization (FISH) testing.

Immunohistochemistry

Tumor blocks were selected after an initial review of hematoxylin and eosin-stained slides to confirm representative tumor lesions. CD44 and CD24 IHC was conducted using the double staining method. Procedures were performed on 4 μm-thick tissue sections using a biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-max automatic slide staining system (Vision BioSystems, Mount Waverley, VIC, Australia), according to the manufacturer's instructions with minor modifications. Briefly, 4 μm-thick formalin-fixed and paraffin-embedded tissue sections were deparaffinized with Bond Dewax solution (Vision BioSystems), and an antigen retrieval procedure was conducted using Bond ER solution (Vision BioSystems) for 30 minutes at 100°C. Endogenous peroxidase was quenched by incubation with hydrogen peroxide for 5 minutes. Sections were incubated for 15 minutes at ambient temperature with a 1 : 6,000 dilution of CD44 primary monoclonal antibody (156-3C11, Neomarkers, Fremont, CA, USA), using the Bond Intense R detection kit (Vision BioSystems). Sections were incubated for 15 minutes at ambient temperature with a 1 : 200 dilution of CD24 primary monoclonal antibody (SN3b, Neomarkers), using the Bond Polymer AP Red detection kit (Vision BioSystems). Nuclei were counterstained with hematoxylin.

Evaluation of immunohistochemical staining

Histological sections were examined by two independent investigators (SJ Lim and JY Sung) blinded to the patient clinical data. All invasive components (100 of 100 IDC cases) and DCIS components (44 of 100 IDC cases) were evaluated separately. Membranes were stained brown and red for CD44 and CD24, respectively. Tumor cells showing brown staining on their membranes in the absence of a distinct red color were counted as CD44+/CD24-/low tumor cells (Fig. 1). An almost entire staining of the individual tumor cell membrane was considered positive. The percentage of IDC CD44+/CD24-/low tumor cells was determined, and tumors with a percentage greater than 20% were considered to have a high prevalence of CD44+/CD24-/low cells. Tumor cells with not only CD44+/CD24-/low expression but also CD44+/CD24+, CD44-/low/CD24+, and CD44-/low/CD24-/low expression profiles were counted. Normal ductal epithelium and DCIS components (if the case had DCIS components) were also counted by the same method, as were 45 other DCIS cases.

Inflammatory cells such as lymphocytes, plasma cells, and endothelial cells of small vessels also showed CD44 or CD24 immunoreactivity.

Statistical analysis

SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical calculations. Receiver operating characteristic (ROC) curve analysis was used to determine the cutoff point for tumor cells with a high prevalence of CD44+/CD24-/low. For each proportion of cells with CD44+/CD24-/low, the sensitivity and specificity for the outcome was plotted to generate an ROC curve. The point on the curve that maximized the sensitivity and specificity of the cell proportions with CD44+/CD24-/low for each clinicopathologic parameter (i.e., with the shortest distance

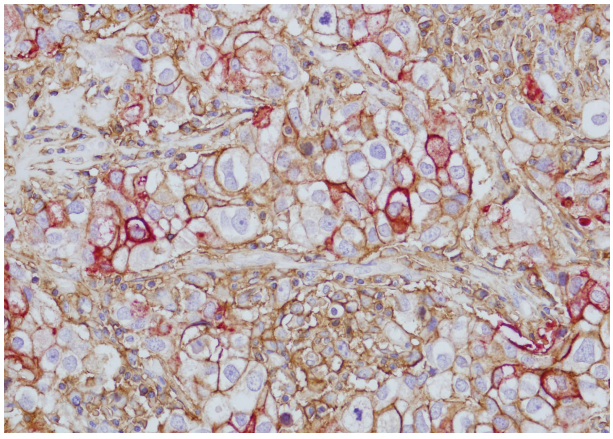

Fig. 1. CD44+/CD24-/low tumor cells. Only brown-colored membranous stained cells are considered CD44+/CD24-/low tumor cells. Approximately 65% of the cells are CD44+/CD24-/low tumor cells. The CD24+ (red color) and CD44-/CD24- (no stain) tumor cells comprised about 15% and 20% of this area, respectively.

to the point [0.0, 1.0]) was selected as the cutoff proportion. Among these, scores yielding the lowest p-value or the greatest area under the curve were selected as the best cutoff proportion. Finally, the cutoff value was determined for the group of tumor cells with a prevalence as high a 20% $CD44^{+}/CD24^{-/low}$ cells. Pearson's χ^2 and Fisher exact tests were used to determine the association between the proportions of tumor cells with $CD44^{+}/CD24^{-/low}$ cells and various clinicopathologic parameters of the patients with IDC and DCIS, respectively. Kaplan-Meier plots were used to estimate the overall survival (OS) of all patients with IDC. The log-rank test was used to compare the OS of the patient subgroups. Survival plots were constructed using the Kaplan-Meier method. The independent t-test was also used to compare means between proportions of $CD44^{+}/CD24^{-/low}$ tumor cells in 45 DCIS cases and DCIS components in 44 IDC. All tests were two-sided. Differences were considered statistically significant when the p-value was < 0.05.

RESULTS

Patient characteristics

The characteristics of the 100 patients with IDC and the 45 patients with DCIS are summarized in Table 1. The characteristics of the 44 patients with DCIS components in IDC (data not shown in Table 1) were as follows: (mean age ± standard deviation) 48.7 ± 8.3 years (range, 32.0 to 66.0 years); tumor size, 2.3 ± 0.7 cm (range, 0.7 to 4.5 cm), hormonal receptor expression status, 32 (72.7%) for ER and 38 (86.4%) for PR; Her2/Neu expression, 17 (38.6%); lymph node (LN) involvement status (pN stage), 17, 17, 8, and 0 for pN0 to 3; Van-Nuys group, 15, 5, and 24 for groups 1 to 3. Seven patients died. No significant difference in histologic grade or ER expression status was observed between the DCIS components of 44 IDC and the 45 DCIS cases.

Proportions of $CD44^{+}/CD24^{-/low}$ tumor cells in breast cancer

The proportions of cells with CD44 and CD24 expression profiles ($CD44^{+}/CD24^{+}$, $CD44^{+}/CD24^{-/low}$, $CD44^{-/low}/CD24^{+}$, and $CD44^{-/low}/CD24^{-/low}$) of separated areas of normal, DCIS & invasive components in 100 IDC cases and those of normal and DCIS in the 45 DCIS cases are summarized in Table 2. The overall

Table 1. Patients characteristics

Characteristics	IDC (100 females)	DCIS (45 females)
Age[a] (yr)	49.3 ± 9.0 (23.0-71.0)	46.7 ± 8.8 (31.0-73.0)
Tumor size[a] (cm)	3.1 ± 1.8 (0.5-11.0)	3.0 ± 2.7 (0.2-9.0)
Overall survival[a] (mo)	58.2 ± 21.5 (7.0-103)	39.2 ± 23.7 (6.0-95.0)
ER expression	71 (71.1%)	33 (73.3%)
PR expression	76 (76.0%)	35 (77.8%)
Her2/Neu expression	34 (34.0%)	21 (46.7%)
pN status[b]		
N0	43	45
N1	31	0
N2	15	0
N3	3	0
Metastasis	8	
TNM stage		
I	16	
IIa, IIb	30, 18	
IIIa, IIIb, IIIc	17, 0, 3	
IV	8	
Grade[c]		
1	30	
2	52	
3	18	
Van-Nuys group		
1		16
2		9
3		20
Death	13	0

[a]Data shown as median ± standard deviation (range); [b]Eight patients of IDC did not undergo axillary lymph node dissection; [c]Modified Bloom-Richardson system.
IDC, invasive ductal carcinoma; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PR, progesterone receptor; Her2/Neu, human epidermal growth factor receptor 2; TNM, tumor, node and metastasis.

Table 2. The proportions of CD44 and CD24 expression profiles at separate areas in invasive ductal carcinoma (IDC) and ductal carcinoma *in situ* (DCIS)

Expression profile	Areas of IDC cases			Areas of DCIS only cases	
	Normal	DCIS component of IDC	IDC	Normal	DCIS
$CD44^{+}/CD24^{+}$ (%)	0.1 ± 0.7	0.7 ± 3.0	0.9 ± 3.6	0.0 ± 0.0	0.7 ± 2.2
$CD44^{+}/CD24^{-/low}$ (%)	12.1 ± 13.6	20.5 ± 21.9	14.5 ± 20.8	10.0 ± 10.0	45.8 ± 32.4
$CD44^{-/low}/CD24^{+}$ (%)	0.0 ± 0.3	0.3 ± 0.8	0.7 ± 3.3	0.0 ± 0.0	0.5 ± 1.2
$CD44^{-/low}/CD24^{-/low}$ (%)	87.8 ± 14.0	80.3 ± 23.6	83.8 ± 23.0	90.1 ± 10.0	52.7 ± 32.0

Data shown as mean value ± standard deviation.

Table 3. Comparison of the ductal carcinoma *in situ* (DCIS) CD44+/CD24-/low tumor cells between the DCIS and DCIS components of invasive ductal carcinoma (IDC)

	DCIS	DCIS component of IDC	p-value[a]
Proportion of CD44+/CD24-/low tumor cells (%)	44.8 ± 31.8	20.5 ± 21.9	< 0.001

Data shown as mean value ± standard deviation.
[a]A p-value was calculated by independent t-test.

proportion of CD44+/CD24-/low tumor cells was 12.0 ± 17.1% for the invasive component of the 100 IDC cases, and 16.7 ± 20.3% for the DCIS components of the 44 cases among the 100 IDC cases. When the 100 IDC cases were classified according to the prevalence of CD44+/CD24-/low tumor cells, 23 of 100 (23.0%) showed > 20% CD44+/CD24-/low tumor cells (high prevalence) in IDC. The 45 cases of DCIS showed a proportion of 44.8 ± 31.8% of CD44+/CD24-/low tumor cells. Forty-four patients had both invasive and DCIS components among the 100 IDC cases. The CD44+/CD24-/low tumor cell proportion of the DCIS components of IDC and the its invasive component were not significantly different (p = 0.546). However, when the CD44+/CD24-/low tumor cell proportions of the DCIS components in these 44 IDC cases were compared with the 45 DCIS cases, significantly more CD44+/CD24-/low tumor cells were present in the DCIS cases than in the DCIS components of IDC (p < 0.001) (Table 3).

Correlation between clinicopathological factors and proportion of CD44+/CD24-/low tumor cells

Table 4 summarizes the relationships between the proportion of CD44+/CD24-/low tumor cells in IDC and patient clinicopathologic factors. In the IDC cases, a high prevalence of CD44+/CD24-/low tumor cells was significantly associated with large tumor size (p = 0.033), high grade using the modified Bloom-Richardson system (p = 0.017), and ER negativity (p = 0.023). Although it was not statistically significant, metastasis (p = 0.079), negativity of both ER and Her2/Neu (basal-like feature, p = 0.057), and high mitosis grade (p = 0.077) were also detected frequently in the high prevalence group. However, LN involvement (p = 0.685), advanced tumor, node and metastasis (TNM) stage (III-IV, p = 0.385), PR expression (p = 0.789), and survival status (p = 0.500) were not significantly associated with tumors that had a high prevalence of CD44+/CD24-/low cells. No statistically significant association was observed between tumors with a high prevalence of CD44+/CD24-/low cells in DCIS and clinicopathological factors (Table 5).

Table 4. Clinicopathologic correlation of CD44+/CD24-/low tumor cells in invasive ductal carcinoma

Characteristics	No. of patients (%)		
	< 20% (n = 77)	≥ 20% (n = 23)	p-value
Age (yr)			
< 50	41 (77.4)	12 (22.6)	0.928[a]
≥ 50	36 (76.6)	11 (23.4)	
Tumor size (cm)			
≤ 5	70 (80.5)	17 (19.5)	**0.033**[a]
> 5	7 (53.8)	6 (46.2)	
LN involvement[c]			
Absent	34 (79.1)	9 (20.9)	0.685[a]
Present	37 (75.5)	12 (24.5)	
Metastasis			
Absent	73 (79.3)	19 (20.7)	0.079[b]
Present	4 (50.0)	4 (50.0)	
TNM stage[c]			
I-II	51 (79.7)	13 (20.3)	0.385[a]
III-IV	20 (71.4)	8 (28.6)	
Mitosis grade			
1-2	66 (80.5)	16 (19.5)	0.077[a]
3	11 (61.1)	7 (38.9)	
Grade			
1-2	67 (87.1)	15 (18.3)	**0.017**[a]
3	10 (55.6)	8 (44.4)	
ER			
Negative	18 (62.1)	11 (37.9)	**0.023**[a]
Positive	59 (83.1)	12 (16.9)	
PR			
Negative	18 (75.0)	6 (25.0)	0.789[a]
Positive	59 (77.6)	17 (22.4)	
Her2/Neu[d]			
Negative	44 (75.9)	14 (24.1)	0.803[a]
Positive	25 (73.5)	9 (26.5)	
Basal-like feature[e]			
Absent	69 (80.2)	14 (19.8)	0.057[a]
Present	8 (57.1)	6 (42.9)	
Death			
No	68 (78.2)	19 (21.8)	0.500[b]
Yes	9 (69.2)	4 (30.8)	

Bold letters represent statistically significant.
[a]Pearson χ^2 test; [b]Fisher's exact test; [c]Lymph node dissection was not done in eight cases; [d]Eight cases have no available tissue to do additional study; [e]Basal-like feature is determined by no immunoreactivity for both ER and Her2/Neu.
LN, lymph node; TNM, tumor, node and metastasis; Grade, Modified Bloom-Richardson system; ER, estrogen receptor; PR, progesterone receptor; Her2/Neu, human epidermal growth factor receptor 2.

Table 5. Clinicopathologic correlation of $CD44^{+}/CD24^{-/low}$ tumor cells in ductal carcinoma *in situ*

Characteristics	No. of patients (%)		
	< 20% (n = 11)	≥ 20% (n = 34)	p-value
Age (yr)			
< 47	5 (21.7)	18 (78.3)	0.666[a]
≥ 47	6 (27.3)	16 (72.7)	
Tumor size (cm)			
≤ 5	9 (25.7)	26 (74.3)	1.000[b]
> 5	2 (20.0)	8 (80.0)	
Van-Nuys group			
1-2	5 (20.0)	20 (80.0)	0.438[a]
3	6 (30.0)	14 (70.0)	
ER			
Negative	4 (33.3)	8 (66.7)	0.448[b]
Positive	7 (21.2)	26 (78.8)	
PR			
Negative	4 (40.0)	6 (60.0)	0.228[b]
Positive	7 (20.0)	28 (80.0)	
Her2/Neu[c]			
Negative	8 (40.0)	12 (60.0)	0.085[b]
Positive	3 (14.3)	18 (85.7)	
Basal-like feature[d]			
Absent	9 (22.5)	31 (77.5)	0.582[b]
Present	2 (40.0)	3 (60.0)	

[a]Pearson χ^2 test; [b]Fisher's exact test; [c]Four cases have no available tissue to do additional study; [d]Basal-like feature is determined by no immunoreactivity for both ER and Her2/Neu.
ER, estrogen receptor; PR, progesterone receptor; Her2/Neu, human epidermal growth factor receptor 2.

Survival and prognosis analysis

Kaplan-Meier survival curves and log-rank tests revealed significantly shorter OS times in patients with advanced TNM stage (III-IV, p = 0.001), metastasis (p < 0.001), older age (≥ 50-years-of-age, p = 0.017), and negative ER expression (p = 0.032). Tumors with a high prevalence of $CD44^{+}/CD24^{-/low}$ cells had no prognostic value (mean OS in the high vs low prevalence group, 89.1 months vs 90.7 months, p = 0.529) (Fig. 2). High grade using the Modified Bloom-Richardson system (grade 3, p = 0.115), LN involvement (p = 0.085), tumor size (> 5 cm, p = 0.207), PR expression (p = 0.296), Her2/Neu expression (p = 0.252), extensive intraductal carcinoma (p = 0.576), and peritumoral inflammation (p = 0.245) did not markedly influence OS.

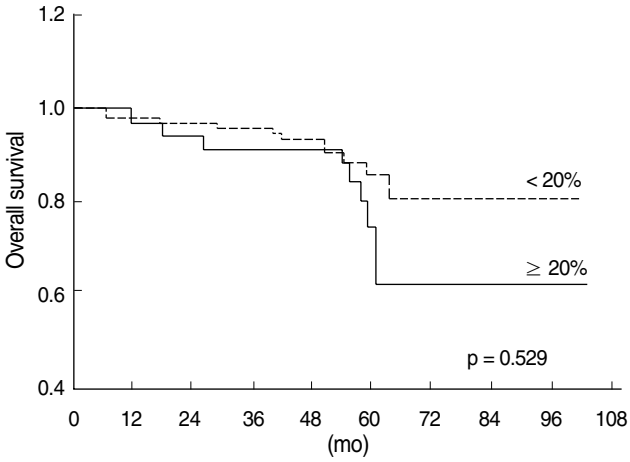

Fig. 2. Prognostic value of a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells in invasive ductal carcinoma. A high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells shows no significant prognostic value for invasive ductal carcinoma.

DISCUSSION

In this study, tumors with a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells in IDC were significantly associated with poor prognostic factors, such as larger mass (p = 0.033), higher grade (p = 0.017), and ER (p = 0.023). Although no statistical significance was found, metastasis (p = 0.079), basal-like features (p = 0.057), and high mitosis grade (p = 0.077) were frequently observed in tumors with a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells in IDC, but OS was not significantly associated with the proportion of $CD44^{+}/CD24^{-/low}$ tumor cells. Similar results showing a correlation between a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells and metastasis, but not of shorter OS, have been reported.[8]

In a study comparing gene expression and genetic profiles of $CD44^{+}$ and $CD24^{+}$ cells isolated from tumors and normal breast tissue, $CD44^{+}$ cells (known as stem cell markers) were predicted to be more invasive and angiogenic than $CD24^{+}$ cells.[9] Besides, CD24 mRNA expression is low in invasive breast cancer cell lines compared with non-invasive cell lines.[10] These observations could explain our result that tumors with a preponderance of $CD44^{+}/CD24^{-/low}$ tumor cells tended to metastasize more frequently.

This study revealed a high prevalence of $CD44^{+}/CD24^{-/low}$ in tumor cells, which was correlated with negative ER status in breast cancer. This result is not surprising, given that stem cells are characterized by a self-renewal capacity and represent a prior stage of differentiation. However, two investigations using mouse mammary stem cells showed contradictory results; ER and PR expression in potential mammary stem cells in one study and no ER or PR expression in a basal population enriched in mouse mammary stem cells in the other.[11,12] Another study involving 136 human breast cancer specimens found no association between

CD44+/CD24-/low tumor cells and ER and PR expression.[8] In a study undertaken to explain this discrepancy of ER and PR expression and the stem cell population, breast cancers were classified by ER expression into type 1 (stem cell origin, ER negative, undifferentiated histology), type 2 (stem cell origin, ER heterogeneous, intermediately differentiated histology), and type 3 (ER positive progenitor cell origin, ER positive, differentiated histology).[13] According to these authors, because adult stem cells are slowly dividing and long-living with high proliferative capacity, they are able to undergo multiple mutations during carcinogenesis. So, different molecular signatures and clinical behaviors such as ER or PR status can be generated by mutations in normal stem or progenitor cell populations. In this study, similar to type 1 breast cancer according to the classification of Dontu *et al*.,[13] about half of IDCs with a high prevalence of CD44+/CD24-/low tumor cells were ER negative, and all ER negative IDCs with a high prevalence of CD44+/CD24-/low tumor cells had high histologic grades. Despite the limitations for applying this classification to our cases, the finding that CD44+/CD24-/low tumor cells appeared to have a tendency for ER negativity is consistent with previous observations.[13]

We also compared the proportion of CD44+/CD24-/low tumor cells between the DCIS components of IDC and DCIS. To our knowledge, this comparison is entirely novel. Differences in the proportion of CD44+/CD24-/low tumor cells between DCIS components of IDC and DCIS was statistically significant, while the proportion of CD44+/CD24-/low tumor cells in IDC (12.4 ± 16.5%) and its DCIS component (15.7 ± 18.2%) was not, suggesting that the natural biological characteristics of pure DCIS and DCIS components might differ. Although this study is limited by the small number of cases, the finding of a higher prevalence of CD44+/CD24-/low tumor cells in DCIS, which is a precursor lesion for invasive breast cancer, could be a reflection of the increased contribution of CD44+/CD24-/low tumor cells in an early step of tumorigenesis. A recent study utilizing a human-in-mouse transplanted model concluded that various human DCIS subtypes appeared to contain distinct subpopulations of tumor initiating cells.[14] We hypothesize that CD44+/CD24-/low tumor cells could be one of the subpopulations of tumors initiating cells in DCIS, but no specific molecular or IHC evidence for tumor initiating cells in DCIS currently exists. However, one study reported a correlation between CD44+/CD24-/low tumor cells and distant metastasis, in which BCSCs related not only to tumor initiation but also tumor progression.[8] Based on our result of similar proportions of CD44+/CD24-/low tumor cells in the DCIS components and invasive components in IDC, the role of the former appeared to be involved in cancer progression rather than cancer initiation, which was different from that in pure DCIS.

As mentioned above, our observations suggest that the high prevalence of CD44+/CD24-/low tumor cells contributes to tumor initiation and metastasis. However, the varying manifestations of tumor progression and clinical behavior in breast cancer cannot be simply explained by the presence of CD44+/CD24-/low tumor cells, although tumors with a high prevalence of CD44+/CD24-/low cells were correlated with aggressiveness, such as metastasis and negative ER status. The results of a study conducted by Abraham *et al*.[8] and that of the current study suggest that the proportion of CD44+/CD24-/low tumor cells failed to demonstrate prognostic value. We hypothesize that a possible reason for this failure is the great complexity of tumorigenesis, which entails heterogeneity of tumor cell phenotypes and a diversity of predictive factors for tumor progression and therapeutic responsiveness. Therefore, it is thought that breast cancer carcinogenesis could be better understood when it is considered not only in terms of the CSC hypothesis, but also in the context of epigenetic events and the surrounding microenvironment.

Recent reports have suggested that the heterogeneity of CSC phenotypes provides clues to explain the absence of a correlation between CD44+/CD24-/low tumor cells and OS in patients with breast cancer. Sheridan *et al*.[15] reported that many basal-type mammary carcinoma cell lines contain CD44+/CD24-/low tumor cells, whereas luminal cell lines do not. Similar to that report, we also found a tendency between the IDC group, with a high prevalence of CD44+/CD24-/low cells, and the absence of both ER and Her2/Neu, which is suggestive of a basal-like breast cancer immunophenotype. However, the difference was not statistically significant. Furthermore, the use of mouse BRCA1 knockout models has provided evidence for the existence of heterogeneous CSC populations (CD44+/CD24-/low tumor cells and CD133 expression).[16] When aggregate information about CSC heterogeneity is obtained in the future, it may be possible to elucidate a more precise role for CD44+/CD24-/low tumor cells.

Studies concerning CSCs have become a subject of attention, because stem cells tend to be more resistant to chemotherapeutics than differentiated cells.[17] This difference may be related to high expression levels of anti-apoptotic proteins or ATP-binding cassette transporters, such as the multi-drug resistance gene.[18-20] Therefore, CSCs can also be envisioned as resistant to chemotherapy. Indeed, it has been demonstrated that CD44+/CD24-/low BCSCs are more resistant to radiation.[21] More frequent negative hormonal receptor status and these findings concerning

BCSCs may explain recurrence and distant metastasis after conventional therapy. Therefore, to devise more effective treatment, additional studies are required to clarify the characteristics and clinicopathologic value of breast cancer stem cells.

In this study, we determined that IDC with a high prevalence of $CD44^{+}/CD24^{-/low}$ tumor cells correlated with aggressive features, such as ER negativity and higher grade, although these features had no prognostic value, similar to Abraham *et al*.[8] This concordance suggests that it may be difficult to understand breast cancer carcinogenesis through the CSC hypothesis of $CD44^{+}/CD24^{-/low}$ tumor cells alone. We also found that the proportion of $CD44^{+}/CD24^{-/low}$ tumor cells in the DCIS components of IDC and DCIS was significantly different. This finding implies that the role of $CD44^{+}/CD24^{-/low}$ tumor cells in DCIS might be different whether it exists in pure DCIS or not.

Although our study had some limitations, the results could contribute to a further understanding of clinical data concerning $CD44^{+}/CD24^{-/low}$ tumor cells in breast cancer, especially the comparison of these cells in pure DCIS and DCIS components of IDC. More studies with other BCSC phenotypes and $CD44^{+}/CD24^{-/low}$ tumor cells are needed to validate the clinicopathologic significance and prognostic value of breast cancer stem cells.

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