SOX2 and ALDH1 as Predictors of Operable Breast Cancer

HIROAKI SHIMA[1], GORO KUTOMI[1], FUKINO SATOMI[1], HIDEKI MAEDA[1], YOSHIHIKO HIROHASHI[2], TADASHI HASEGAWA[3], MITSURU MORI[4], TOSHIHIKO TORIGOE[2] and ICHIRO TAKEMASA[1]

[1]*Department of Surgery, Surgical Oncology and Science, Sapporo Medical University, Sapporo, Japan;*
[2]*Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan;*
[3]*Department of Surgical Pathology, Sapporo Medical University, Sapporo, Japan;*
[4]*Department of Public Health, Sapporo Medical University, Sapporo, Japan*

Abstract. *Aim: Sex-determining region Y-box binding protein-2 (SOX2) and aldehyde dehydrogenase-1 (ALDH1) are known cancer stem-cell markers, and represent candidate predictors for breast cancer prognosis. In this study we investigated the relationships between SOX2/ALDH1 expression and prognosis. Materials and Methods: One hunred and two breast cancer surgical specimens were immunohistochemically analyzed for SOX2 and ALDH1 expression. Results: Disease-free survival (DFS) and overall survival (OS) were significantly poorer for SOX2-positive patients than SOX2-negative (p=0.0024 and p=0.0021, respectively), and for ALDH1-positive patients than ALDH1-negative (p=0.0049 and p=0.0083). DFS and OS were worse for SOX2- or ALDH1-positive patients than double-negative (p=0.0053 and p=0.0166). While an obvious tendency toward worse DFS was seen for estrogen receptor (ER)-negative patients, and attenuated for ER-positive, only SOX2/ALDH1 any-positive patients showed significantly poorer DFS (p=0.0258). Conclusion: SOX2 and ALDH1 can be considered markers of poor prognosis, particularly in ER-negative patients. SOX2/ALDH1 any-positivity might also offer a reliable predictor of poor prognosis.*

Sex-determining region Y-box binding protein-2 (SOX2) is a transcription factor essential to the maintenance of the pluripotent stem cell state in embryonic stem cells and induced pluripotent stem cells (1, 2). SOX2 has recently been discovered to be aberrantly expressed in cancer cells, including those of the lungs, brain, ovaries, bone, colon, upper urinary tract, skin, and breasts (3-5). On the other hand, expression of aldehyde dehydrogenase-1 (ALDH1) is well known as a marker of cancer stem cells associated with the stem-like properties of self-renewal in breast cancer (6, 7). In the physiological state, ALDH1 is well known as a cytosolic enzyme responsible for the metabolism of intracellular aldehydes (8, 9). Both SOX2 and ALDH1 have thus been identified as stem cell markers, but SOX2 and ALDH1 might reflect different aspects of the stem cell nature.

Through clinical investigations, SOX2 and ALDH1 have been correlated with poor prognosis in breast cancer (6, 7, 10). Furthermore, various mechanisms allow cancer stem-like cells to show greater resistance to chemo- and radiotherapy than non-cancer stem-like cells, suggesting that existence of these cells is a prognostic factor in cancer patients (4, 11). SOX2 and ALDH1 are, therefore, seen as markers expressed in both normal and cancer stem cells, including breast cancer (12). These potentially prognostic factors have been reported to reflect different clinico-pathological features (13, 14). This study investigated whether SOX2 and ALDH1 are associated with prognosis and whether relationships exist between these markers and different clinicopathological features.

Materials and Methods

We retrospectively analyzed a group of 102 patients with operable primary breast cancer diagnosed and treated at the Sapporo Medical University Hospital. All tissue samples were derived from a series of consecutive cases at the Department of Surgical Pathology within the same Institution, and clinicopathological factors of these patients were analyzed. The age of patients ranged from 31 to 85 years, and all patients were diagnosed between January 2011 and December 2012.

Immunohistochemistry and scoring. Sections (4 mm) of formalin-fixed, paraffin-embedded tumor specimens were immunostained after heat-induced epitope retrieval in citrate buffer (pH 6.0) using an autoclave with a polyclonal antibody against SOX2 (dilution

Correspondence to: Hiroaki Shima, Department of Surgery, Surgical Oncology and Science, Sapporo Medical University, S 1, W 16, Chuo-ku, Sapporo, 060-8543, Japan Tel: +81 116112111, Fax: +81 116131678, e-mail: simahiro@sapmed.ac.jp

Key Words: SOX2, ALDH1, breast cancer, predictive marker, cancer stem cells.

1:100; Abnova, Taipei, Taiwan) and monoclonal antibody against ALDH1 (clone 44/ALDH, dilution 1:3000; BD Bioscience, Franklin Lakes, NJ, USA). A sensitized agent from a Bond Polymer Refine Detection kit (Leica Biosystems, Tokyo, Japan) was used in immunohistochemistry for ALDH after the first antibody reaction. Subsequent incubations with a secondary biotinylated antibody, avidin-conjugated peroxidase complex and chromogen were performed on a Bond Polymer Refine Detection kit. Slides were then counterstained with hematoxylin, rinsed, dehydrated through graded alcohols into a non-aqueous solution, and cover-slipped with mounting media.

All specimens were reviewed independently using light microscopy in at least ten areas at ×400 magnification by an investigator. For SOX2, comparatively obvious nuclear staining was considered positive in any cancer cell (10, 15). Diffuse cytosol staining without nuclear staining in cancer cells was not defined as a positive result. Positive stromal ALDH1 expression was defined as the existence of more than 10% positive stromal cells within the tumor tissue (Figure 1) (16).

Hormone receptor and HER2 expression. Breast cancer specimens were divided into 4 groups through immunohistochemistry (IHC) for estrogen receptor (ER) and human epidermal growth factor receptor type 2 (HER2). Tumor cells showing >10% positive staining were considered ER-positive. HER2 status was determined by IHC or by fluorescence *in situ* hybridization (FISH) analysis. HER2-positive results were defined as 3+ on IHC (>10% of cells showing membrane staining) or as positive by FISH (with amplification ratio >2.0 indicating a positive status) in accordance with the American Society of Clinical Oncology/College of American Pathologists Guidelines (17). Similarly, a progesterone receptor (PgR)-positive result was defined as >10% positively stained tumor cells.

Statistical analysis. We tested the relationships between SOX2 or ALDH1 and other clinicopathological parameters, specifically the pathological T stage, lymph node status, nuclear grade and lymphovascular invasion, using the Chi-square method or t method. Disease-free survival (DFS) and overall survival (OS) were assessed using the Kaplan-Meier method, and differences between the two groups were compared using the Wilcoxon test. Uni- and multivariate regression analyses with Cox proportional hazards regression modeling, with DFS or OS as the dependent variable, were used to evaluate expression of SOX2 or ALDH1 as potential independent prognostic factors. A value of $p=0.05$ was considered to indicate statistical significance. Calculations were performed using JMP11 software (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics. Median age was 60.5 years. Among 102 patients, 81 patients had a diagnosis of invasive ductal carcinoma and 19 patients were diagnosed with other type of invasive breast cancer. Median duration of follow-up was 30.3 months. Among the 102 patients, 16 patients had experienced a recurrence of the disease, and 12 patients died during the 4-year observation period.

Relationship between SOX2/ALDH1 expression and clinicopathological factors. SOX2-positive cells were

Table I. *Background and association of cancer stem cell marker staining with clinicopathological featured of operable breast cancer.*

		n=102	SOX2$^+$ (n=9)		SOX2$^-$ (n=93)			ALDH1$^+$ (n=17)			ALDH1$^-$ (n=84)		
Age	Median	60.5	52	32-85	61	31-85	p-NS	63	31-85		60	32-85	*p*=NS
Menopausal status	pre-/post-	35/71	4/5	44.4/55.6%	29/64	31.2/68.8%	*p*=NS	6/11	35.3/64.7%		27/57	32.1/67.9%	*p*=NS
Tumor size	T1	60	4	44.4%	56	60.9%	*p*=0.0126	11	64.7%		49	59.0%	
	T2	33	3	33.3%	30	32.6%		4	23.5%		28	33.7%	
	T3<	8	2	22.2%	6	6.5%		2	12.8%		6	7.3%	
Lymph node metastatic status	1</0	26/76	4/5	44.4/55.6%	22/71	23.7/76.3%	*p*=NS	6/11	35.3/64.7%		20/68	22.7/77.3%	*p*=NS
Histology	IDC	81	9	100%	74	79.6%	*p*=NS	12	70.6%		70	83.3%	
	other	19	0	0%	19	20.4%		5	29.4%		14	16.7%	
ER	pos/neg	80/22	6/3	66.7/33.3%	74/19	80.6/20.4%	*p*=NS	13/4	76.5/23.5%		66/18	78/6/21.4%	*p*=NS
PgR	pos/neg	61/40	4/5	44.4/55.6%	57/35	62.0/38.0%	*p*=NS	9/8	17.7/82.3%		51/32	61.5/38.5%	*p*=NS
HER2	pos/neg	13/88	3/6	33.3/66.7%	10/82	10.9/89.1%	*p*=NS	3/14	37.5/62.5%		10/73	12.1/87.9%	*p*=NS
ly	pos/neg	33/62	4/5	44.4/55.6%	29/57	33.7/66.3%	*p*=NS	7/8	46.7/53.3%		26/53	32.9/67.1%	*p*=NS
NG	3/1-2	31/64	7/2	77.8/22.2%	24/62	27.9/72.1%	*p*=0.0085	4/13	23.5/76.5%		27/51	34.6/ 65.4%	*p*=NS
Ki67	median	20.0	40.0	1-90	20.0	1-80	*p*=0.0101	20.0	5-80		20.0	1-90	*p*=NS
Subtype	ER+/HER2–	75	5	55.6%	70	76.1%	*p*=NS	12	70.6%		62	74.7%	*p*=NS
	ER+/HER2+	4	1	11.1%	3	3.3%		1	5.9%		3	3.6%	
	ER–/HER2–	14	2	22.2%	12	13.0%		2	11.7%		12	14.5%	
	ER–/HER2+	8	1	11.1%	7	7.6%		2	11.7%		6	7.2%	

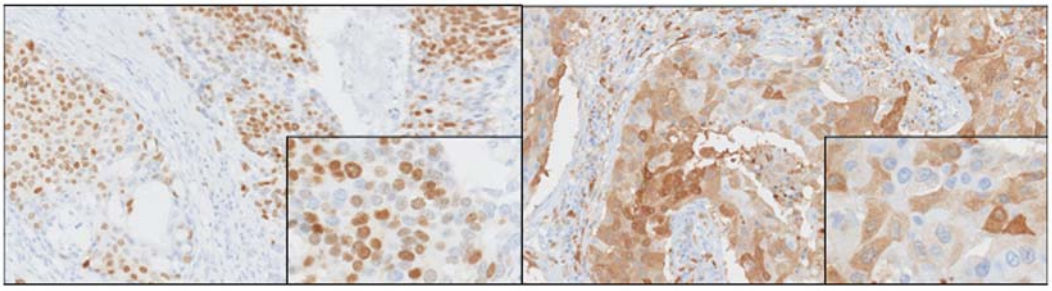

Figure 1. *Immunohistological staining of SOX2 and ALDH1 in breast cancer. Left panel a SOX2-positive case is shown. In this case, nucleus cancer cells were distinctly stained by anti-SOX2 polyclonal antibody. And we defined SOX2 positive as any distinctly stained nucleus. Right panel an ALDH1 positive case is shown. In this case, stromal ALDH1 expression was heterogeneously seen in a cancer nest. Positive stromal ALDH1 expression was defined as more than 10% of positive stromal cells within the tumor tissue.*

observed in 9 of 106 patients (8.8%), as shown in Table I. SOX2 expression correlated with tumor size (p=0.0126), higher nuclear grade (NG; p=0.0085) and higher percentage of Ki67 (p=0.0101). On the other hand, ALDH1 expression was observed in 17 of 102 patients. No significant relationship was seen between ALDH1 expression and these clinicopathological factors, Different characteristics in patients' background such as tumor size, NG and Ki67 were seen between SOX2- and ALDH1-positivity.

Of particular interest, 10 or more metastatic lymph nodes at the time of diagnosis were seen in 2 patients (22.2%) in the SOX2-positive group, and 3 patients (3.1%) in the SOX2-negative group, while the number of patients who had 1 or more metastatic lymph nodes at diagnosis was not significantly different between the two groups. Although no significant difference in lymph node metastatic status (more than one node) was evident, there were significantly more patients with 10 or more metastatic lymph nodes in the SOX2-positive group than in the SOX2-negative group (OR=8.95; 95% confidence interval (CI)=1.28-62.74, p=0.0096), while no significant difference in the frequency of patients with over 10 metastatic nodes was seen between ALDH1-positive and -negative patients (OR=3.78; 95%CI=0.58-24.55, p=not significant).

SOX2 and ALDH1 expression might be related to prognosis. A significant decrease in DFS was seen in SOX2-positive patients compared to SOX2-negative patients (p=0.0024, Figure 2A). A significant difference was also seen in ALDH1-positive patients compared with ALDH1-negative patients (p=0.0049, Figure 2B). Moreover, DFS was significantly poorer in SOX2- or ALDH1-positive patients than in SOX2 and ALDH1 double-negative patients (p=0.0053, Figure 2C). SOX2 and ALDH1 were both positive in only 1 patient. SOX2-positive, ALDH1-negative results were seen in 8 patients, while ALDH1-positive, SOX2-negative results were seen in 16 patients.

OS was also significantly poorer in SOX2-positive patients than in SOX2-negative patients (p=0.0021, Figure 3A). Similarly, a significant decrease in OS was seen in ALDH1-positive patients compared to ALDH1-negative positive (p=0.0073, Figure 3B). Moreover, OS was significantly poorer in patients with SOX2- or ALDH1-positive results than when SOX2 and ALDH1 were both negative (p=0.0166, Figure 3C).

SOX2 and ALDH1 expression might show different relationships with the distribution of ER status. In ER-negative patients, SOX2-positive, ALDH1-positive and SOX2/ALDH1 double-positive results were associated with significantly poor DFS (p=0.0081, p=0.0092 and p=0.0499, respectively; Figure 4A-C). Although ER-positive patients showed no significant difference in DFS between SOX2 and ALDH1 (Figure 4D and E), any combination of SOX2- or ADH1-positive (SOX2/ALDH1 any-positive) patients showed significantly inferior DFS than SOX2/ALDH1 double-negative patients (p=0.0258; Figure 4F).

OS showed a similar tendency, with SOX2-positive, ALDH1-positive and SOX2/ALDH1 double-positive results among ER-negative patients indicating significantly poorer prognosis (p=0.0013, p=0.0048 and p=0.028; Figure 5A-C). Conversely, ER-positive patients showed no significant differences between SOX2-positive, ALDH1-positive and SOX2/ALDH1 any-positive results (Figure 5D-F).

SOX2 and ALDH1, either individually or in combination, may predict poor prognosis. In univariate analyses, tumor size, presence or absence of node metastasis, NG, Ki67, SOX2-positive and SOX2/ALDH1 any-positive were significantly associated with DFS (Table II). In multivariate analyses for DFS, no significant difference was seen between clinicopathological factors selected from univariate analyses. Even if calculations in multivariate analysis included SOX2/ALDH1 any-positive cases, no significant associations

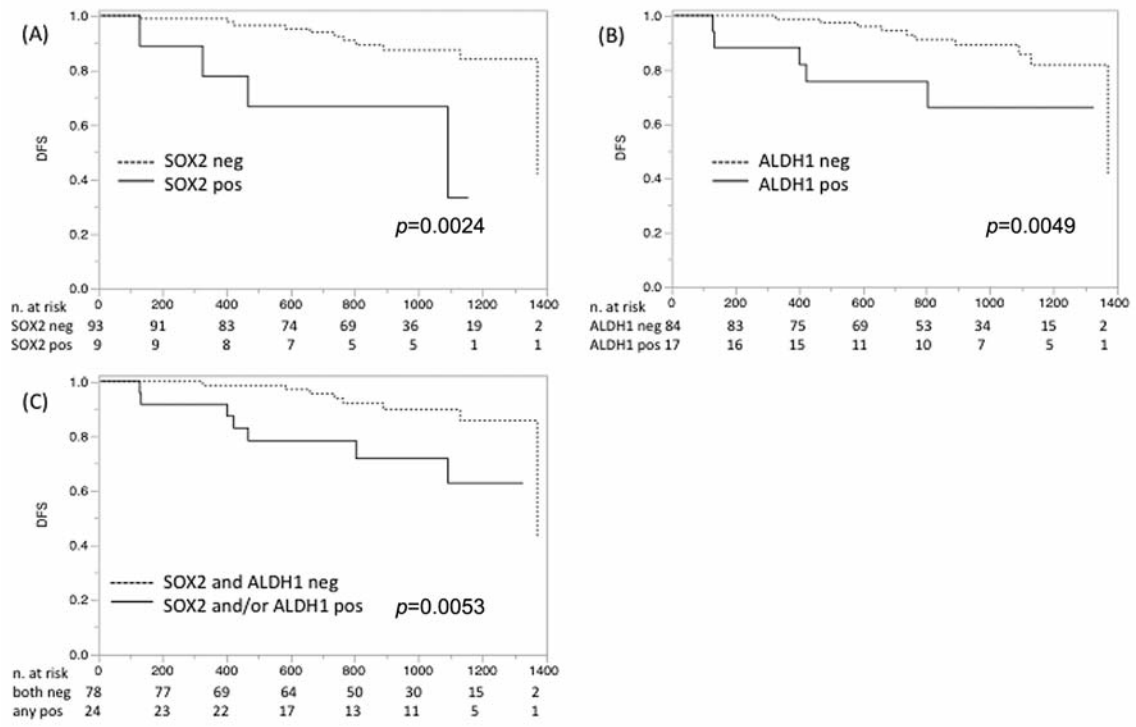

Figure 2. *Kaplan-Meier curves for disease-free survival according to (A) SOX2 expression status, (B) ALDH1 expression status and (C) combination of SOX2 and ALDH1 status, dividing into two groups: SOX2 and/or ALDH1 any-positive versus neither SOX2- nor ALDH1-negative.*

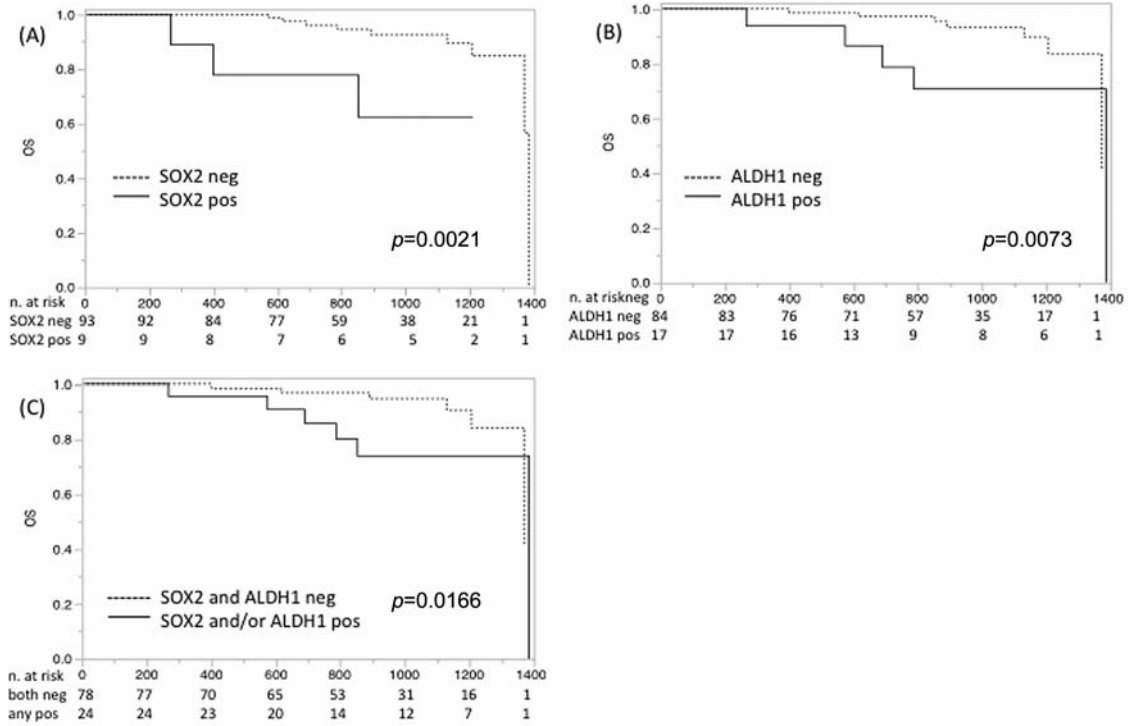

Figure 3. *Kaplan-Meier curves for overall survival according to (A) SOX2 expression status, (B) ALDH1 expression status and (C) combination of SOX2 and ALDH1 status.*

Table II. *Prognostic factors for disease free survival in univariate and multivariate analyses.*

		Univariate analysis			Multivariate analysis		
		HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age		4.02	0.031-2.01	0.189			
Menopausal status	pre/post	2.7	0.96-7.79	0.059			
Tumor size	2/1	3.76	1.13-14.38	0.031	1.37	0.27-7.01	0.698
	3/1	7.62	1.50-34.63	0.017	18.38	0.69-250.05	0.074
Lymph node metastatic status	pos/neg	4.8	1.67-14.60	0.0042	3.53	0.68-19.42	0.133
Histology	other/IDC	1.05	0.24-3.38	0.94			
ER	neg/pos	1.8	0.55-5.14	0.31			
PgR	neg/pos	1.06	0.38-3.17	0.92			
HER2	pos/neg	2.97	0.77-9.11	0.11			
iy	pos/neg	2.5	0.77-8.05	0.12			
NG	3/1-2	3.11	1.07-9.49	0.037	1.73	0.16-16.19	0.639
Ki67		19.5	2.41-142.21	0.0069	3.08	0.10-118.79	0.102
SOX2	pos/neg	5.17	1.41-15.56	0.0162	1.58	0.19-11.61	0.653
ALDH1	pos/neg	3.11	0.95-9.05	0.059			
SOX2orALDHl	pos/neg	3.55	1.21-10.37	0.022			

Table III. *Prognostic factors for overall survival in univariate and multivariate analyses.*

		Univariate analysis			Multivariate analysis		
		HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (years)		22.18	0.003-0.56	0.0163	2.73	0.23-38.05	0.665
menopausal status	pre/post	3.79	1.12-14.70	0.0328	20.12	0.36-5863.15	0.148
Tumor size	2/1	8.31	1.95-57.36	0.0034	9.13	0.44-354.01	0.15
	3/1	5.9	0.65-52.58	0.107	64.18	0.67-20575.82	0.072
Lymph node metastatic status	pos/neg	7.5	2.01-34-6	0.0021	3.56	0.16-90.61	0.41
Histology	other/IDC	1.04	0.16-4.16	0.96			
ER	neg/pos	3.14	0.90-10.51	0.0716			
PgR	neg/pos	1.84	0.55-6.41	0.314			
HER2	pos/neg	4.24	1.08-14.39	0.0396	1.99	0.084-53.2	0.65
ly	pos/neg	5.48	1.42-26.36	0.0137	6.96	0.54-213.53	0.14
NG	3/1-2	3.46	0.99-13.55	0.0524			
Ki67		16.24	1.28-179.63	0.033	1.25	61.66-0.80	0.91
SOX2	pos/neg	5.08	1.09-18.39	0.0398	1.27	0.053-30.13	0.88
ALDH1	pos/neg	2.62	0.68-8.83	0.152			
SOX2 or ALDH1	pos/neg	2.51	0.72-8.38	0.141			

were identified (p=0.42, 95%CI, 0.37-9.69). Similarly, in univariate analyses, age, menopausal status, tumor size, nodal status, HER2, ly, Ki67 and SOX2 were significantly associated with OS (Table III). In multivariate analyses for OS, no significant differences were found in any clinicopathological factors, which were identified by univariate analyses.

Discussion

Recent reports have noted that SOX2 and ALDH1 expressions are associated with prognosis in breast cancer (6, 7, 10). Although these stem cell markers have singularly been used to assess prognosis in individualistic ways, no investigations appear to have reached any final determination on the impact of these markers in combination, nor have any comparisons been made. We investigated both the prognoses of SOX2 and ALDH1 individually and the relationship between each stem-cell marker and breast cancer prognosis. A relationship has been reported between stem cell markers (SOX2 and ALDH1) and clinicopathological factors, suggesting that some differences exist between SOX2 and ALDH1 for those factors, as described below.

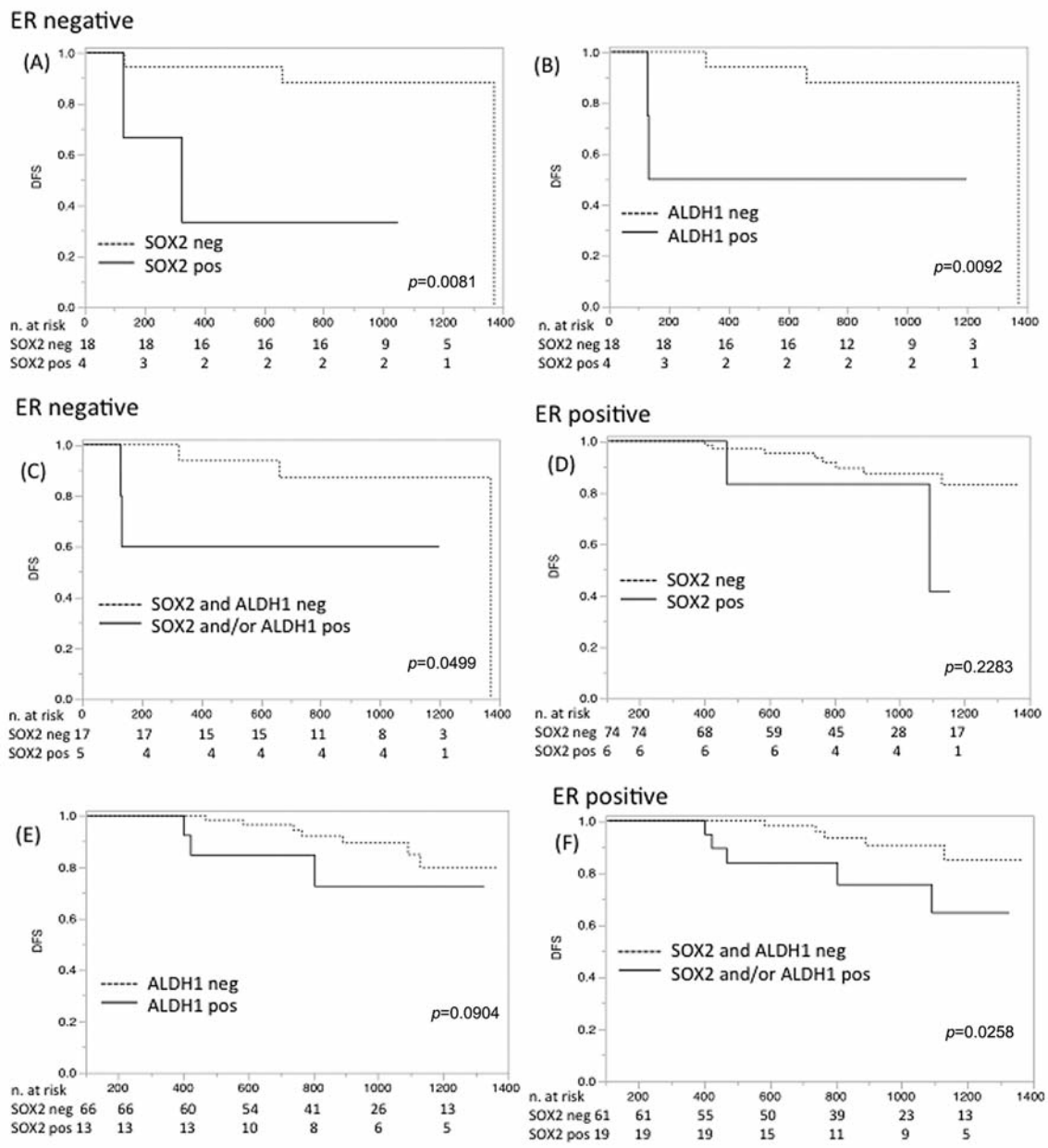

Figure 4. *For disease-free survival Kaplan-Meier curves were drawn in a distribution of ER status respectively, according to (A, D) SOX2 expression status, (B, E) ALDH1 expression status and (C, F) SOX2/ALDH1 any positive status. They were divided into A-C in ER-negative patients and D-F in ER positive patients.*

Ginestier *et al*. reported that ALDH1-positive tumors were associated with high histological grade, HER2 overexpression, and absence of ER and PR expression, and no correlations were found with age, tumor size, or lymph node metastasis (14). On the other hand, Lengerke *et al*. reported that high expression of SOX2 might be associated with larger tumor size and positive lymph node status (10). Other investigators have reported an increased SOX2 expression rate with an increase in pN stage in node-positive cancers, while no significant association of SOX2 expression was found with lymph node status (13). A similar phenomenon was seen in the present study. With regard to SOX2, although no relationship was apparent in lymph node metastasis status, of note the patients with 10 or more metastatic lymph nodes were significantly more frequent in the SOX2-positive group than in the SOX2-

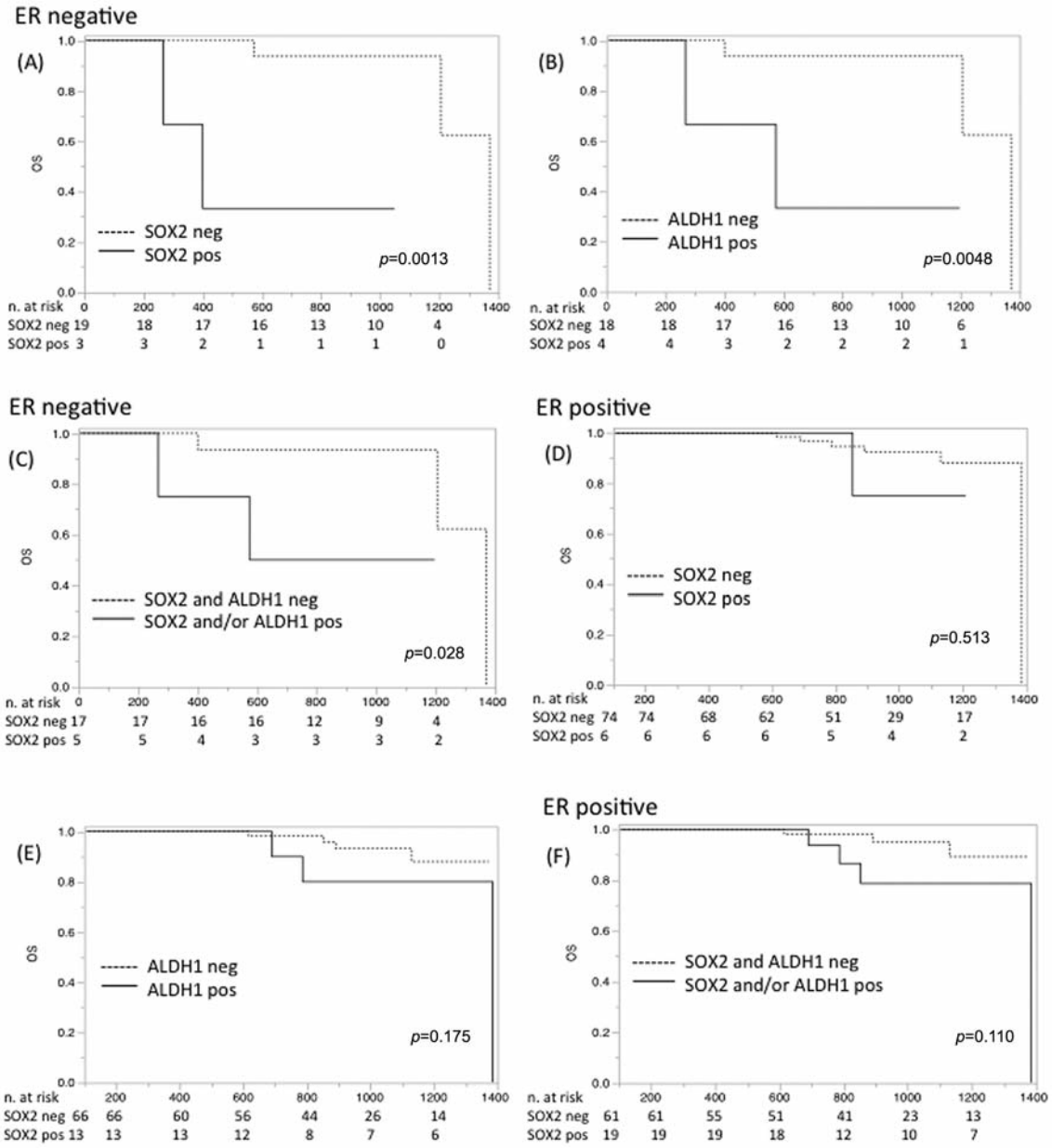

Figure 5. *For overall survival, Kaplan-Meier curves were drawn in a distribution of ER status, according to (A, D) SOX2 expression status, (B, E) ALDH1 expression status and (C, F) SOX2/ALDH1 any-positive status. They were divided into A-C in ER-negative patients and D-F in ER-positive patients.*

negative group, while no significant difference between ALDH1-positive and -negative groups was seen. This suggested that SOX2 might also be strongly correlated with over 10 lymph node metastases compared to ALDH1. Clinically, presence of over 10 metastatic lymph nodes is well known to correlate strongly with poor prognosis (17, 18). This study also suggested that SOX2 expression might be expected when lymph node metastatic involvement is determined to be over 10. Thus, differences in relationships to clinicopathological factors between SOX2 and ALDH1 appear consistent with our findings. In this study, SOX2 and ALDH1 statuses were likely to be associated with different backgrounds. SOX2 expression was significantly associated with tumor size, NG and Ki67, while ALDH1 expression was not significantly associated with any clinicopathological factors.

The fact that HER2 overexpression increased and Ki67 index was at a higher level in SOX2-positive patient backgrounds is likely to result in a proportionate decrease in ER-positive and HER2-negative patients (19,20). Our study substantiates the concept that overexpression of SOX2 reduces ER expression and increases the number of stem cells, and is thus associated with poor prognosis (21).

SOX2 and ALDH1 expression was significantly associated with poor DFS and OS. A significant difference was also seen in comparisons between SOX2/ALDH1 any-positive and double-negative results. SOX2, ALDH1 and the combination of those markers are likely to become useful candidates as markers of poor prognosis. Particularly in ER-negative patients, SOX2, ALDH1 and SOX2/ALDH1 any-positive results might become predictors of poor prognosis in terms of DFS and OS. By contrast, among ER-positive patients, only SOX2/ALDH1 any-positive results were significantly associated with poor DFS, but no other markers showed significant differences in DFS or OS. The possibility remains that late recurrences may have been missed in the relatively short observation period in this study, because both DFS and OS curves indicated two lines (SOX2/ALDH1 any-positive vs. double-negative) separating later for ER-positive patients than for ER-negative patients. However, no factors (including age, menopausal status, nodal status, HER2, Ki67 and SOX2) were significant in multivariate analysis for DFS or OS. The finding that many factors were associated with SOX2 might explain why SOX2 expression was independently associated with DFS on univariate analysis, but not on multivariate analysis. The same reason may also explain the difference between univariate analysis and multivariate analysis findings for SOX2/ALDH1 any-positive.

Of note, positive results for both SOX2 and ALDH1 were only seen in one case, a 38-year-old woman with T3N3M0 stage IIIC triple-negative breast cancer with a Ki-67 index of 80%, who received neoadjuvant chemotherapy resulting in progressive disease. Her cancer grew rapidly and local recurrence and multiple bone metastases were diagnosed 129 days postoperatively, and she died on postoperative day 267 with multiple liver metastases. The one case with positive results for both SOX2 and ALDH1 was resistant to chemotherapy and showed rapid deterioration.

Positivity beyond SOX2 or ALDH1 alone is likely to represent an important detail. If one of the two markers has been assessed, SOX2 and ALDH1 might reflect diverse mechanisms and are likely to have impact as a predictor of poor prognosis in breast cancer. Those stemness markers might contribute largely to prediction of poor prognosis and modification treatment intensity especially in ER-negative patients. Thus, immunohistochemical assessment of both SOX2 and ALDH1 might offer a reliable tool. In conclusion, both SOX2 and ALDH1 might be useful predictors of poor prognosis. Combination assessment for SOX2 and ALDH1 any-positivity might also be reliable as a predictor of poor prognosis. Those characteristic features might have potential clinical utility, and further clinical investigation is needed to clarify the details.

References

1 Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *25*: 663-676, 2006.

2 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell *131*: 861-872, 2007.

3 Kitamura H, Torigoe T, Hirohashi Y, Asanuma H, Inoue R, Nishida S, Tanaka T, Fukuta F, Masumori N, Sato N and Tsukamoto T: Prognostic impact of the expression of ALDH1 and SOX2 in urothelial cancer of the upper urinary tract. Mod Pathol *26*: 117-124, 2013.

4 Hirohashi Y, Torigoe T, Inoda S, Takahashi A, Morita R, Nishizawa S, Tamura Y, Suzuki H, Toyota M and Sato N: Immune response against tumor antigens expressed on human cancer stem-like cells/tumor-initiating cells. Immunotherapy *2*: 201-211, 2010.

5 Michifuri Y, Hirohashi Y, Torigoe T, Miyazaki A, Kobayashi J, Sasaki T, Fujino J, Asanuma H, Tamura Y, Nakamori K, Hasegawa T, Hiratsuka H and Sato N: High expression of ALDH1 and SOX2 diffuse staining pattern of oral squamous cell carcinomas correlates to lymph node metastasis. Pathol Int *62*: 684-689, 2012.

6 Ohi Y, Umekita Y, Yoshioka T, Souda M, Rai Y, Sagara Y, Sagara Y, Sagara Y and Tanimoto A: Aldehyde dehydrogenase 1 expression predicts poor prognosis in triple-negative breast cancer. Histopathology *59*: 776-780, 2011.

7 Ito M, Shien T, Omori M, Mizoo T, Iwamoto T, Nogami T, Motoki T, Taira N, Doihara H and Miyoshi S: Evaluation of aldehyde dehydrogenase 1 and transcription factors in both primary breast cancer and axillary lymph node metastases as a prognostic factor. Breast Cancer *23*: 437-444, 2016.

8 Ikawa M, Impraim CC, Wang G and Yoshida A: Isolation and characterization of aldehyde dehydrogenase isozymes from usual and atypical human livers. J Biol Chem *258*: 6282-6287, 1983.

9 Alamgeer M, Ganju V and Kumar B *et al*: Changes in aldehyde dehydrogenase-1 expression during neoadjuvant chemotherapy predict outcome in locally advanced breast cancer. Breast Cancer Res *16*: R44, 2014.

10 Lengerke C, Fehm T, Kurth R, Neubauer H, Scheble V, Müller F, Schneider F, Petersen K, Wallwiener D, Kanz L, Fend F, Perner S, Bareiss PM and Staebler A: Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma. BMC Cancer *28*: 42, 2011.

11 Chen Y, Shi L, Zhang L, Li R, Liang J, Yu W, Sun L, Yang X, Wang Y, Zhang Y and Shang Y: The molecular mechanism governing the oncogenic potential of SOX2 in breast cancer. J Biol Chem *283*: 17969-17978, 2008.

12 Jung K, Wang P, Gupta N, Gopal K, Wu F, Ye X, Alshareef A, Bigras G, McMullen TP, Abdulkarim BS and Lai R: Profiling gene promoter occupancy of Sox2 in two phenotypically distinct breast cancer cell subsets using chromatin immunoprecipitation and genome-wide promoter microarrays. Breast Cancer Res *16*: 470, 2014.

13 Huang YH, Luo MH, Ni YB, Tsang JY, Chan SK, Lui PC, Yu AM, Tan PH and Tse GM: Increased SOX2 expression in less differentiated breast carcinomas and their lymph node metastases. Histopathology *64*: 494-503, 2014.
14 Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS and Dontu G: ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell *1*: 555-567, 2007.
15 El-Maqsoud NM and Abd El-Rehim DM: Clinicopathologic implications of EpCAM and Sox2 expression in breast cancer. Clin Breast Cancer *14*: e1-9, 2014.
16 Ohi Y, Umekita Y, Yoshioka T, Souda M, Rai Y, Sagara Y, Sagara Y, Sagara Y and Tanimoto A: Aldehyde dehydrogenase 1 expression predicts poor prognosis in triple-negative breast cancer. Histopathology *59*: 776-780, 2011.
17 Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM and Hayes DF: American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med *131*: 18-43, 2007.
18 Koca E, Kuzan TY, Dizdar O, Babacan T, Sahin I, Ararat E and Altundag K: Outcomes of locally advanced breast cancer patients with ≥ 10 positive axillary lymph nodes. Med Oncol *30*: 615, 2013.
19 Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, Cuzick J, Houghton J, Williams N, Mallon E, Bishop H, Ellis I, Larsimont D, Sasano H, Carder P, Cussac AL, Knox F, Speirs V, Forbes J and Buzdar A: Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. J Clin Oncol *26*: 1059-1065, 2008.
20 Inic Z, Zegarac M, Inic M, Markovic I, Kozomara Z, Djurisic I, Inic I, Pupic G and Jancic S: Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information. Clin Med Insights Oncol *8*: 107-11, 2014.
21 Simões BM, Piva M, Iriondo O, Comaills V, López-Ruiz JA, Zabalza I, Mieza JA, Acinas O and Vivanco MD: Effects of estrogen on the proportion of stem cells in the breast. Breast Cancer Res Treat *129*: 23-35, 2011.

Received April 2, 2016
Revised May 9, 2016
Accepted May 17, 2016