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EPIDEMIOLOGY

Serum CD44 levels and overall survival in patients with HER2-positive breast cancer

Jong-Min Baek · Quanri Jin · Joe Ensor · Delphine R. Boulbes · Francisco J. Esteva

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Abstract CD44 is an adhesion molecule involved in tumor cell invasion and metastasis. The function of CD44 in breast cancer is not understood completely, or is its role as a predictive or prognostic factor. In this study, we tested for the hypothesis that the concentration of soluble CD44 (sCD44) in serum is correlated with clinicopathological factors, especially HER2, and survival in patients with breast cancer. We retrospectively identified 110 patients with breast cancer who had been treated at The University of Texas MD Anderson Cancer Center (MDACC) from September 2001 to May 2004. Sera were collected before definitive surgery in patients with stage I or II breast cancer, before initiation of neoadjuvant chemotherapy (if indicated) for patients with stage I–III breast cancer, and before initiation of systemic therapy in patients with stage IV breast cancer. sCD44 levels were determined using an enzyme-linked immunosorbent assay. The median age at diagnosis was 51 years (range, 28.6–87.1 years). sCD44 concentration was correlated with tumor stage ($P = 0.0308$). sCD44 serum concentration did not predict pathological response in patients treated with neoadjuvant chemotherapy. Among patients with distant metastases, sCD44 levels were significantly higher in patients with liver involvement than in patients with metastases at other sites. The overall survival rate did not differ between patients with high sCD44 concentration and patients with low sCD44 concentration. However, sCD44 concentration was a significant predictor of overall survival for patients with HER2-positive breast cancer, while no difference in overall survival rates was observed in patients with HER2-negative breast cancer. To the best of our knowledge, this is the first study to show an association between circulating sCD44 levels and survival in HER2-positive breast cancer patients. Our results suggest a role for sCD44 as a prognostic marker. Furthermore, sCD44 level may offer a new clinical therapeutic target in HER2-positive breast cancer.

Keywords Breast cancer · Soluble CD44 · HER2 · Survival

J.-M. Baek · Q. Jin · D. R. Boulbes · F. J. Esteva (✉)
Department of Breast Medical Oncology, Unit 1354,
The University of Texas MD Anderson Cancer Center,
1515 Holcombe Blvd, Houston, TX 77030, USA
e-mail: fjesteva@mdanderson.org

J. Ensor
Department of Biostatistics, Unit 1411, T. Boone Pickens Academic Tower, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

Introduction

CD44 is a cell surface adhesion molecule that plays an important role in the interaction between cancer cells and their microenvironments during invasion and metastasis. Recently, CD44 was highlighted as a cancer stem cell marker [1]. CD44 is composed of an extracellular amino-terminal domain, a transmembrane domain, and a carboxyl-terminal cytoplasmic tail. The proximal extracellular domain binds hyaluronan and is responsible for the alternative splicing of several isoforms of CD44. The standard form of CD44 (CD44std) is widely distributed, whereas other isoforms are expressed in a more restricted fashion. CD44std and its variants, except variants v8–v10, have the potential ability to bind hyaluronan [2].

Proteolytic ectodermal cleavage of CD44 results in the release of soluble extracellular CD44 (sCD44) and the subsequent release of a CD44 intracellular domain, which is considered as a key event in activation of the

CD44-signaling pathway [3]. Extracellular cleavage of CD44 is highly prevalent in various malignancies, including gliomas, breast cancers, lung cancers, colon cancers, gastric cancers, and ovarian carcinomas [4, 5]. It has been suggested that sCD44 acts as a competitive inhibitor of the hyaluronan–CD44 interaction [3]; however, the role of sCD44 generated via ectodermal cleavage remains unclear [6].

Clinically, elevated sCD44 is associated with poor outcome and disease progression in hematological malignancies, such as non-Hodgkin's lymphoma and B-cell lymphoma [7, 8]. The CD44 variants v5 and v6 are expressed in tumor tissues of breast cancer; however, the relationship between CD44 overexpression and other clinicopathological factors remains undefined [9–11]. Upregulations of sCD44std or its variants have been associated with larger tumor size and lymph node metastasis [12] and with distant metastasis and higher stage [13] in breast cancer patients.

The expressions of CD44 or its variants in patients' primary tumors tend to positively correlate with the level of its soluble forms in the serum [6, 14, 15]. Overexpression of the CD44 protein in primary breast cancer tissues has been significantly associated with advanced stage and poor overall survival [16]. Although sCD44 is considered as a possible survival marker in many hematologic malignancies and in some solid tumors [17, 18], its ability to predict survival in breast cancer has not been fully addressed.

The *HER2* gene, which is a member of the epidermal growth factor receptor family, is amplified in about 20–25% of cases of human breast cancer and is associated with aggressive phenotype and metastasis [19]. HER2 overexpression has been reported to increase the proportion of stem cells in mammary carcinomas and in normal mammary epithelial cells [20]. The administration of HER2 inhibitors such as trastuzumab or lapatinib has led to clinical benefits in HER2-positive breast cancer patients, possibly because these inhibitors can target the cancer stem cell population in HER2-positive tumors [20]. Recent studies of neoadjuvant therapy demonstrated an increase in the proportion of CD44+/CD24–breast cancer stem cells after chemotherapy; however, targeting HER2 with lapatinib reduced the cancer stem cell population, resulting in an increased rate of pathological complete responses [21]. Despite the suggesting that CD44 and HER2 positivity are both negative prognostic factors, it is still unclear whether the two are related to one another.

On the basis of unpublished laboratory data, we hypothesized that patients with HER2-positive breast cancer have higher circulating sCD44 levels than patients with HER2-negative breast cancer and that serum sCD44 can thus serve as a prognostic factor in breast cancer patients. To test this hypothesis, we retrospectively analyzed the levels of total sCD44 in the sera of patients with breast cancer and tested its potential as a novel prognostic marker relative to HER2 status.

Materials and methods

Patients and samples

This study was approved by the Institutional Review Board of MDACC, and all the patients had signed an informed consent document at the time of blood collection. We identified 110 women who had been treated for breast cancer at MDACC between September 2001 and May 2004. These patients had donated a blood sample for identification of prognostic marker, and serum was stored for future research. We identified 56 patients with HER2-positive breast cancer, and matched them with 54 patients with HER2-negative breast cancer. Their serum samples had been obtained before definitive surgery or before initiation of neoadjuvant chemotherapy, if indicated, in patients with stages I–III breast cancer and before systemic therapy for metastatic disease in patients with stage IV breast cancer. No patient had received trastuzumab. All the samples had been immediately frozen at −80°C. Data regarding patient demographics (ethnicity, age, and menopausal status) and primary tumor histopathology (tumor size, lymph node metastasis, TNM stage, histologic grade, nuclear grade, hormone receptor status, HER2 status, and Ki-67) were obtained by reviewing the patients' medical records.

Enzyme-linked immunosorbent assay

Soluble CD44std (sCD44std) was quantified using an enzyme-linked immunosorbent assay according to the manufacturer's instructions (Abnova Corporation, Taipei City, Taiwan), and all the measurements were performed in triplicate. The sCD44std (human) enzyme-linked immunosorbent assay kit, a sandwich enzyme immunoassay, detects all circulating CD44 isoforms that include the standard protein sequence. In brief, the wells of 96-well microtiter plates were precoated with an anti-human sCD44std antibody. Then, the serum was diluted to a 1:30 concentration in sample diluent added to the wells. The sCD44 molecules present in the diluted serum bound to the antibodies absorbed onto each well. Then, a horseradish peroxidase-conjugated monoclonal antibody against sCD44 was added to the wells, and the wells were incubated at room temperature for 3 h. After incubation, unbound enzyme-conjugated antibodies were removed by washing three times with wash buffer, and 100 ul of substrate solution was added to each well. The

coloring reaction was terminated by the addition of stop solution, and absorbance was measured at 450 nm on a Victor™ *X*3 multilabel plate reader (PerkinElmer Inc., Shelton, CT, USA). To determine the levels of sCD44, we constructed standard curves using serially diluted CD44 antigens and then compared them with the experimental samples.

Statistical analysis

Statistical analyses of relationships between sCD44std levels and other patient and tumor data were performed using SAS Release 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). These analyses included calculation of descriptive statistics, hypothesis testing, and survival analysis. Descriptive statistics of demographic data included means, medians, and 95% confidence intervals. Medians and confidence intervals of the sCD44 concentration data were calculated, and *P* values for differences between groups were determined by fitting the data to mixed-effects linear models to account for the dependent nature of the repeated measurements from each patient. The within-subject correlation coefficient for the sCD44 concentration was estimated to be 0.96. Values for continuous variables were dichotomized and analyzed using Fisher's z-transformation for Pearson's product-moment correlation coefficient. Overall and recurrence-free survival analyses included log-rank tests of significance and Kaplan–Meier estimates of survival. Univariate and multivariate Cox proportional hazards models were used for producing hazard rate estimates. Survival analyses were based on the within-patient averages of sCD44 concentration. The level of statistical significance was defined as $P < 0.05$.

Results

The median age at diagnosis was 51 years (range, 28.6–87.1 years). The cohort was composed of 74 Caucasian patients (67.3%), 14 African-American patients (12.7%), 7 Asian/Pacific patients (6.4%), and 15 Hispanic patients (13.6%). There were no significant differences in age at the time of serum sampling between ethnicity groups. Another patient characteristic, menopausal status is shown in Table 1.

Serum levels of sCD44

The median sCD44 concentration was 415.5 ng/ml (range, 154.9–1237.7 ng/ml), and there were no significant differences in sCD44 concentration according to age ($P = 0.1501$) or ethnicity ($P = 0.0506$).

Serum sCD44 levels in subgroups of patients defined according to tumor characteristics are shown in Table 1. There were no differences in sCD44 concentration in subgroups dichotomized according to hormone receptor status ($P = 7634$), triple negativity status ($P = 0.3797$), or HER2 positivity status ($P = 0.1485$). sCD44 concentration did differ significantly between subgroups defined according to tumor stage ($P = 0.0308$); the strength of the significance increased when stage was dichotomized as I/II and III/IV ($P = 0.0038$). There was a significant correlation between Ki-67 level and sCD44 concentration (Pearson correlation coefficient, $r = 0.34$; $P = 0.0136$); however, Ki-67 levels were measured in only 53 patients.

Pathological response in patients receiving neoadjuvant chemotherapy

Fifty-four of the 110 patients included in this study received neoadjuvant chemotherapy. The mean concentrations of sCD44 were 430.1 ng/ml in the 12 patients who experienced a pathological complete response and 427.6 ng/ml in the 42 patients who did not experience a pathological complete response (not significant).

Correlation between sCD44 levels and the location of distant metastasis

Forty-seven of the 110 patients included in this study experienced a distant metastasis. sCD44 levels were significantly higher in patients with distant liver metastases than in patients with distant metastases to other sites (Table 2).

Survival analyses based on sCD44 concentration

The average serum concentration of sCD44 in each patient in the cohort was analyzed for its utility as a predictor of survival time. Overall survival and recurrence-free survival were defined as the number of months between the sCD44 serum sample collection and one of the following events: death, loss to follow-up, or recurrence. The median follow-up period was 28.9 months (range, 1.4–111.9 months). The median follow-up period for the 43 patients who had died was 35.8 months (range, 1.4–97.5 months), and the median follow-up period for the 67 censored patients was 88.0 months (range, 32.2–111.9 months).

Overall survival as a function of sCD44 concentration

A Cox proportional hazards model using sCD44 concentration as a continuous variable revealed that sCD44 level had a significant effect on overall survival ($P = 0.0026$) and on age-adjusted overall survival ($P = 0.0113$). To

Table 1 sCD44 concentration by patient and tumor characteristics

Characteristic	Patients (N)	sCD44 concentration (ng/ml)						*P* value
		Median	Mean	95% CI bounds		Minimum	Maximum	
				Lower	Upper			
Tumor size								
≤2 cm	38	427.6	431.0	385.9	476.2	154.9	812.2	0.8226
>2 cm	72	409.3	437.4	404.5	470.2	210.8	1237.7	
Lymph node metastasis*								
No	54	422.1	427.6	394.8	460.3	154.9	769.6	0.7313
Yes	44	407.9	436.0	399.7	472.4	225.8	1042.0	
Stage								
I	16	407.9	400.8	333.4	468.2	154.9	613.5	0.0308
II	42	395.3	398.5	356.9	440.1	210.8	724.3	
III	20	444.9	456.9	396.6	517.2	233.2	1042.0	
IV	32	443.4	487.0	439.3	534.7	242.5	1237.7	
HER2 status								
−	54	408.9	415.5	378.0	453.1	154.9	752.3	0.1485
+	56	417.4	454.1	417.3	491.0	210.8	1237.7	
Hormone receptor								
−	34	429.9	441.2	393.5	489.0	233.2	1042.0	0.7634
+	76	411.0	432.5	400.5	464.4	154.9	1237.7	
Nuclear grade								
I	2	302.2	311.3	117.2	505.4	256.4	381.4	0.1918
II	35	393.3	410.2	363.8	456.6	154.9	1237.7	
III	72	431.7	448.1	415.8	480.5	210.8	1042.0	
Triple negative								
No	93	416.1	440.2	411.4	469.1	154.9	1237.7	0.3797
Yes	17	404.4	407.6	340.3	475.1	242.5	752.3	
Menopausal status								
Pre-	39	393.3	401.6	357.7	445.5	225.8	812.2	0.1615
Post-	71	432.8	453.6	421.1	486.2	154.9	1237.7	
Entire cohort	110	415.5	435.2	408.7	461.6	154.9	1237.7	

* Of the 110 patients in this study; only 98 patients had a known nodal status because of no harvested lymph node due to metastatic disease

Table 2 sCD44 concentration in 47 breast cancer patients by site of distant metastasis

Site of metastasis	Patients (*N*)	sCD44 Concentration (ng/ml)						*P* value
		Median	Mean	95% CI bounds		Minimum	Maximum	
				Lower	Upper			
Bone								
No	38	413.3	460.9	405.9	515.9	242.5	1237.7	0.4139
Yes	9	374.4	409.4	296.3	522.5	247.3	752.3	
Lung								
No	36	403.7	453.2	396.2	510.1	242.5	1237.7	0.8771
Yes	11	431.1	444.1	341.1	547.1	335.6	724.3	
Liver								
No	39	400.4	425.8	374.3	477.4	242.5	812.2	0.0211
Yes	8	515.3	574.1	460.3	687.9	247.3	1237.7	

further explore the effect of sCD44 concentration on overall survival, we divided the patients into subgroups with sCD44 concentrations above and below the median value of the within-patient average, 415.4 ng/ml. We used the Kaplan–Meier method to determine the effect of sCD44 concentration on time-to-event outcome (Fig. 1) [22].

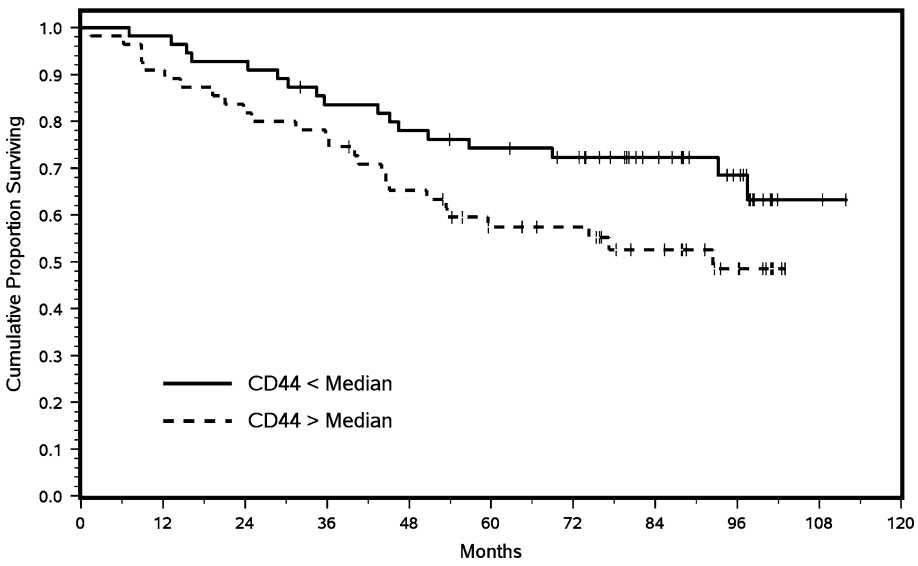

Fig. 1 Overall survival in patients with breast cancer, divided into subgroups according to sCD44 concentration (log-rank test, $P = 0.0559$)

Overall survival according to dichotomized sCD44 concentration did not significantly differ between these two groups, based on the Cox proportional hazards model ($P = 0.0594$). The survival of patients with high levels of sCD44 was worse than that of patients with low levels of sCD44; however, this difference was not significant (Fig. 1).

To investigate the effect of sCD44 concentration on overall survival in patients divided into subgroups according to HER2 status, we analyzed the levels of sCD44 in HER2-positive ($n = 56$) and HER2-negative ($n = 54$) patients independently and divided the data at the medians. In HER2-positive patients, the sCD44 value had a significant effect on overall survival in a Cox proportional hazards model. Figure 2a depicts the Kaplan–Meier survival curve for the HER2-positive patients divided according to sCD44 concentration (median = 421.6 ng/ml). In HER2-positive patients, sCD44 concentration was a significant predictor of survival when used as a continuous or dichotomous variable based on the Cox proportional hazards model (dichotomized data, $P = 0.0438$) and using the log-rank test ($P = 0.0368$). In HER2-negative patients, there was no significant difference in overall survival according to whether the sCD44 concentration was above or below the median (406.2 ng/ml) ($P = 0.8645$) (Fig. 2b) or when it was used as a continuous variable; thus, in HER2-negative patients, sCD44 concentration was not a significant predictor of survival.

Recurrence-free survival as a function of CD44 concentration

Our analysis of recurrence-free survival included all the patients with stages I–III cancer, 39 of whom had HER2-positive disease and 39 HER2-negative disease. Recurrence-free survival according to sCD44 concentration was not significantly different, when used as either a continuous or dichotomized variable, or between subgroups that are divided according to HER2 status. In addition, there were no significant differences in recurrence-free survival between these subgroups including local recurrences ($P = 0.0835$). Multivariate analysis showed that advanced stage (stage III) and nuclear grade III were significant predictors of decreased recurrence-free survival ($P < 0.0016$ and $P = 0.0335$, respectively). However, stepwise selection in the subgroup of patients with HER2-positive cancer revealed that only sCD44 concentration came close to having significant prognostic value, with $P = 0.0510$ for recurrence-free survival during the follow-up period. In the subgroup of patients with HER2-negative breast cancer, hormone receptor positivity and advanced stage were significant predictors of distant recurrence-free survival ($P < 0.0152$ and $P = 0.0001$, respectively).

Discussion

We measured circulating levels of total sCD44 in the sera of breast cancer patients and found a significant correlation between sCD44 concentration and survival in HER2-positive breast cancer. We did not find any difference in sCD44 concentration between subgroups of patients with HER2-positive and HER2-negative breast cancers. We also investigated whether sCD44 concentration is correlated with other clinicopathological factors or a differentiating role in predicting survival in subgroups of patients divided according to their cancers' HER2 status. Overall survival times did not differ between patients with high sCD44 concentration and patients with low sCD44 concentration. However, sCD44 concentration was a significant predictor

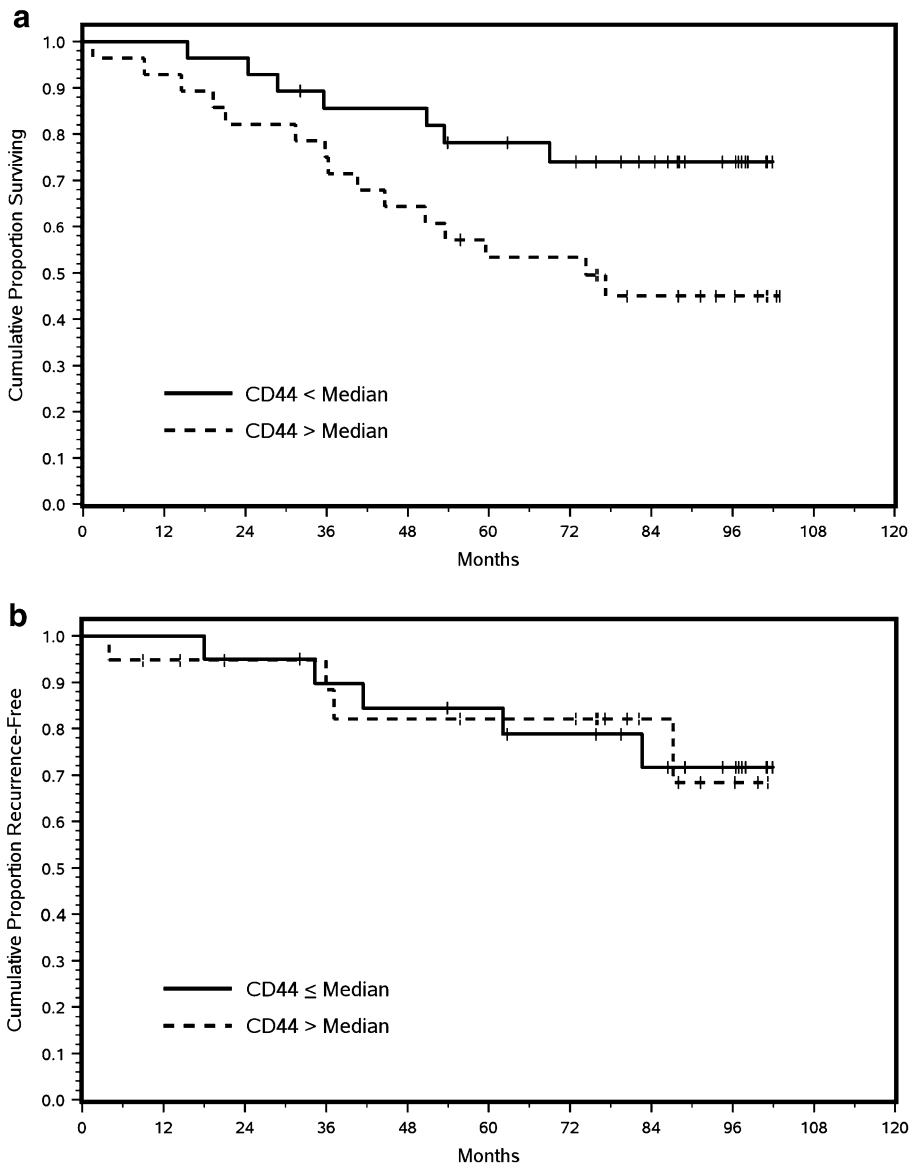

Fig. 2 **a** Overall survival in patients with HER2-positive breast cancer, divided into subgroups according to sCD44 concentration (log-rank test, $P = 0.0368$). **b** Overall survival in patients with HER2-negative breast cancer, divided into subgroups according to sCD44 concentration (log-rank test, $P = 0.8645$)

of overall survival for patients with HER2-positive breast cancer, but not for patients with HER2-negative breast cancer.

The presence of a CD44 variant in a highly metastatic rat pancreatic cancer cell line and the blocking effect of anti-variant CD44 on metastases demonstrated that CD44 is involved in the metastatic process [23, 24]. In addition, overexpression or increased serum levels of CD44 are frequently found in various human malignancies—not only hematologic malignancies, but also solid tumors—indicating the potential of CD44 as a diagnostic marker [12, 25]. However, some studies have shown increased CD44 expression only in patients with metastatic cancer, not in patients without metastatic disease or healthy controls [6, 26].

Although the association between inherited genetic polymorphisms and breast cancer is controversial, it is known that African Americans have a lower incidence of breast cancer but a higher mortality rate than Caucasian Americans. Also, a higher incidence of unique single nucleotide polymorphisms in CD44 intron 1 was reported in African-American breast cancer patients than in Caucasian-American patients [27]. Our current results showed that African-American patients had the highest concentration of sCD44 among the ethnicity groups we studied; however, the difference was not significant.

Overexpression of CD44 or its variants in primary tumor tissues has been correlated with other clinicopathological factors such as larger tumor size and the presence of lymph node metastasis [12, 14]; however, these results have not

always been confirmed [28, 29]. In the present study, we demonstrated that upregulation of sCD44 was correlated with advanced stage in breast cancer patients, as shown in previous studies [30], which suggests that sCD44 plays an important role in tumor progression.

Although sCD44 is thought to be related to tumor burden and disease activity [31], some studies have reported the absence of differences in sCD44 levels before and after surgery [14, 28]. In addition, among subgroups of patients who underwent neoadjuvant chemotherapy, we did not find any significant differences in the serum concentration of sCD44 between those who did and did not achieve a pathological complete response. Thus, according to our results, sCD44 level does not predict treatment response.

Breast carcinomas, like other solid tumors, are composed of heterogeneous tumor cell populations; thus, the correlations between clinicopathological parameters may vary according to subgroup. Consistent with this supposition, the role of CD44 in tumor progression has been reported differentially, depending on the type of cancer and the specific patient cohort. In a recent study, CD44 expression has been found to be correlated with overall survival only in breast cancer patients with hormone-receptor-negative disease [32]. In gastric cancer, sCD44 variant v6 was correlated with clinicopathological parameters only in the diffuse type, not in the intestinal type [33]. In our study, sCD44 levels were prognostic only in the subset of HER2-positive breast cancers.

We found significantly higher sCD44 levels in patients with liver metastases than in patients with other distant metastases. This result is consistent with those of previous reports [6, 26, 33]. However, in many cancers, a high level of CD44 expression has not always been associated with an unfavorable outcome. Many studies have yielded different results with variation depending on the type of cancer, study methodology, or sensitivity of molecular processing.

CD44 is consistently overexpressed in primary breast cancers and cell lines with CD44 amplification [34]. Although one study has reported that the absence of CD44 variant v6 mRNA expression was a significant independent predictor of poor prognosis in breast cancer patients, variant v6 mRNA expression was not correlated with the expression of the corresponding protein [35]. Another group reported that upregulation of the CD44 mRNA was associated with longer recurrence-free survival and overall survival whereas immunohistochemical detection of the CD44 protein was not [36]. Results obtained using two methods, such as analyses of protein expression and mRNA levels, are not always comparable [15]. Differences found in the sensitivity of reverse transcription-polymerase chain reaction method and immunohistochemical stains used, as well as incomplete translation or posttranslational modification of the marker itself may explain the discrepancies between studies.

Conclusion

To our knowledge, this is the first study showing an association between the circulating levels of sCD44 and survival in HER2-positive breast cancer, with high sCD44 levels predicting poor survival. We found that sCD44 plays an important prognostic role in HER2-positive breast cancer, but not in HER2-negative breast cancer. Although this was not a randomized or a large-scale prospective study, we believe that our results point to an important need to clarify the role of sCD44 in HER2-positive breast cancer.

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Conflict of interest None.

References

1. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988
2. Stamenkovic I, Aruffo A, Amiot M, Seed B (1991) The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells. EMBO J 10:343–348
3. Nagano O, Saya H (2004) Mechanism and biological significance of CD44 cleavage. Cancer Sci 95:930–935
4. Okamoto I, Tsuiki H, Kenyon LC et al (2002) Proteolytic cleavage of the CD44 adhesion molecule in multiple human tumors. Am J Pathol 160:441–447
5. Guo YJ, Liu G, Wang X et al (1994) Potential use of soluble CD44 in serum as indicator of tumor burden and metastasis in patients with gastric or colon cancer. Cancer Res 54:422–426
6. Lackner C, Moser R, Bauernhofer T et al (1998) Soluble CD44 v5 and v6 in serum of patients with breast cancer. Correlation with expression of CD44 v5 and v6 variants in primary tumors and location of distant metastasis. Breast Cancer Res Treat 47:29–40
7. Molica S, Vitelli G, Levato D, Giannarelli D, Gandolfo GM (2001) Elevated serum levels of soluble CD44 can identify a subgroup of patients with early B-cell chronic lymphocytic leukemia who are at high risk of disease progression. Cancer 92:713–719
8. Niitsu N, Iijima K (2002) High serum soluble CD44 is correlated with a poor outcome of aggressive non-Hodgkin's lymphoma. Leuk Res 26:241–248
9. Guriec N, Marcellin L, Gairard B et al (1996) CD44 exon 6 expression as a possible early prognostic factor in primary node negative breast carcinoma. Clin Exp Metastasis 14:434–439

10. Lockhart MS, Waldner C, Mongini C et al (1999) Evaluation of soluble CD44 in patients with breast and colorectal carcinomas and non-Hodgkin's lymphoma. Oncol Rep 6:1129–1133
11. Sanchez Lockhart M, Hajos SE, Basilio FM, Mongini C, Alvarez E (2001) Splice variant expression of CD44 in patients with breast and ovarian cancer. Oncol Rep 8:145–151
12. Mayer S, zur Hausen A, Watermann DO et al (2008) Increased soluble CD44 concentrations are associated with larger tumor size and lymph node metastasis in breast cancer patients. J Cancer Res Clin Oncol 134:1229–1235
13. Sheen-Chen SM, Chen WJ, Eng HL, Sheen CC, Chou FF, Cheng YF (1999) Evaluation of the prognostic value of serum soluble CD 44 in patients with breast cancer. Cancer Invest 17:581–585
14. Amirghofran Z, Jalali SA, Hosseini SV, Vasei M, Sabayan B, Ghaderi A (2008) Evaluation of CD44 and CD44v6 in colorectal carcinoma patients: soluble forms in relation to tumor tissue expression and metastasis. J Gastrointest Cancer 39:73–78
15. Woodman AC, Sugiyama M, Yoshida K et al (1996) Analysis of anomalous CD44 gene expression in human breast, bladder, and colon cancer and correlation of observed mRNA and protein isoforms. Am J Pathol 149:1519–1530
16. Bhatavdekar JM, Patel DD, Shah NG et al (2000) Prognostic significance of immunohistochemically localized biomarkers in stage II and stage III breast cancer: a multivariate analysis. Ann Surg Oncol 7:305–311
17. Yamane N, Tsujitani S, Makino M, Maeta M, Kaibara N (1999) Soluble CD44 variant 6 as a prognostic indicator in patients with colorectal cancer. Oncology 56:232–238
18. Kokko LL, Hurme S, Maula SM et al (2011) Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous-cell carcinoma. Oral Oncol 47(6):510–516
19. Esteva FJ, Hortobagyi GN, Sahin AA, Smith TL, Chin DM, Liang SY, Pusztai L, Buzdar AU, Bacus SS (2001) Expression of erbB/HER receptors, heregulin and P38 in primary breast cancer using quantitative immunohistochemistry. Pathol Oncol Res 7(3):171–177
20. Korkaya H, Paulson A, Iovino F, Wicha MS (2008) HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion. Oncogene 27:6120–6130
21. Li X, Lewis MT, Huang J et al (2008) Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 100:672–679
22. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2006) REporting recommendations for tumor MARKer prognostic studies (REMARK). Breast Cancer Res Treat 100:229–235
23. Gunthert U, Hofmann M, Rudy W et al (1991) A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. Cell 65:13–24
24. Seiter S, Arch R, Reber S et al (1993) Prevention of tumor metastasis formation by anti-variant CD44. J Exp Med 177:443–455
25. Masson D, Denis MG, Denis M et al (1999) Soluble CD44: quantification and molecular repartition in plasma of patients with colorectal cancer. Br J Cancer 80:1995–2000
26. Kopp R, Classen S, Wolf H, Gholam P, Possinger K, Wilmanns W (2001) Predictive relevance of soluble CD44v6 serum levels for the responsiveness to second line hormone- or chemotherapy in patients with metastatic breast cancer. Anticancer Res 21:2995–3000
27. Zhou J, Nagarkatti PS, Zhong Y, Creek K, Zhang J, Nagarkatti M (2010) Unique SNP in CD44 intron 1 and its role in breast cancer development. Anticancer Res 30:1263–1272
28. Weg-Remers S, Hildebrandt U, Feifel G, Moser C, Zeitz M, Stallmach A (1998) Soluble CD44 and CD44v6 serum levels in patients with colorectal cancer are independent of tumor stage and tissue expression of CD44v6. Am J Gastroenterol 93:790–794
29. Zalewski B (2004) Levels of v5 and v6 CD44 splice variants in serum of patients with colorectal cancer are not correlated with pT stage, histopathological grade of malignancy and clinical features. World J Gastroenterol 10:583–585
30. Gotte M, Yip GW (2006) Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. Cancer Res 66: 10233–10237
31. Chang SM, Xing RD, Zhang FM, Duan YQ (2009) Serum soluble CD44v6 levels in patients with oral and maxillofacial malignancy. Oral Dis 15:570–572
32. Kim HJ, Kim MJ, Ahn SH et al (2011) Different prognostic significance of CD24 and CD44 expression in breast cancer according to hormone receptor status. Breast 20:78–85
33. Saito H, Tsujitani S, Katano K, Ikeguchi M, Maeta M, Kaibara N (1998) Serum concentration of CD44 variant 6 and its relation to prognosis in patients with gastric carcinoma. Cancer 83: 1094–1101
34. Klingbeil P, Natrajan R, Everitt G et al (2010) CD44 is overexpressed in basal-like breast cancers but is not a driver of 11p13 amplification. Breast Cancer Res Treat 120:95–109
35. Shah NG, Trivedi TI, Vora HH et al (2010) CD44v6 expression in primary breast carcinoma in western India: a pilot clinicopathologic study. Tumori 96:971–977
36. Berner HS, Suo Z, Risberg B, Villman K, Karlsson MG, Nesland JM (2003) Clinicopathological associations of CD44 mRNA and protein expression in primary breast carcinomas. Histopathology 42:546–554