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CD44 Expression in Invasive Breast Carcinoma: Stromal Expression is More Predictor of Prognosis

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Abstract: CD44 is a cell-surface glycoprotein that is expressed in various tissues, including breast carcinoma. It integrates with hyaluronanto regulate cellular signaling, cell adhesion to extracellular matrix (ECM) components and also stimulate a variety of functions leading to breast cancer progression. In this study we investigated the expression of CD44 in breast carcinoma to explore its association with cancer progression.Eighty six cases were immunostained for CD44 and its expression in tumor and stromal cells were evaluated. Twenty eight cases (32.6%) showed positive expression of CD44 in the tumor cells, while thirty cases (34.9%) showed positive immunostaining for CD44 in stromal cells. No statistically significant association was obtained between CD44 expression in tumor cells and any established prognostic factors. CD44 expression in stromal cells was associated with nodal metastasis and advanced stage. CD44 stromal expression was higher among hormone negative cases and HER-2 enriched subtype. Three-year DFS was 59.2% and was not affected by CD44 expression in tumor cells (p=0.800), while worsened by CD44 expression in stromal cells. CD44 negative cases achieved better DFS than CD44 positive cases (69.6% versus 40%; respectively). CD44 expression in stromal cells was also more predictive for 3-year OS than its expression in tumor cells; where there was decline in 3 year OS from CD44 positive cases in relation to CD44 negative cases (56% versus 74.5%; respectively, p=0.057).We concluded that CD44 expression in stromal cells is more associated with tumor aggressiveness and poor patient outcome.

Key words: CD44 · Stromal expression · Breast carcinoma

INTRODUCTION

Breast cancer is the most frequent cancer among women. It is a heterogeneous disease, with distinct morphologies, metastatic behavior and therapeutic response. CD44 is a transmembrane receptor protein that participates in many cell–cell and cell–matrix interactions. The role of CD44 in cancer is complex because alternative splicing of its mRNA leads to production of several CD44 variants, hence, conflicting results have been published on the contribution of CD44 to the progression of human breast cancer [1]. CD44 was identified as the first integral hyaluronan (HA) binding "receptor". HA is one of the main components of the extracellular matrix and its abundance is associated with aggressive tumor type and cancer progression [2]. HAmediatedCD44 signaling has received a great deal of attention in cancer field. Both CD44 and HA are over-expressed/elevated at sites of tumor attachment [3]. HA contributes significantly to cell adhesion, proliferation and migration/invasion. There is also a great deal of evidence linking high level of HA production in human carcinomas to aggressive phenotypes and metastasis, including the progression of breast cancer [4].

The interactions between HA and CD44 can also activate a large number of intracellular signaling pathways [5]. Post-translational modifications of CD44 influence its binding to HA and the resulting signaling. In addition, there are studies indicating that HA binding is essential for proteolytic shedding of the extracellular domain of CD44, which is especially important for promoting the migration of carcinoma cells [6].

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HER2 (the human epidermal growth factor 2) is amplified in 20–25% of the breast cancers and its overexpression can activate signaling pathways promoting cell survival, tumor growth and metastasis. Experiments using cultured cells have shown that CD44 can interact with HER2 and that binding of HA to CD44 modulates this interaction [7]. The high level of active autophosphorylated HER2 found in mammary carcinoma cells is also dependent on endogenous HA–CD44 interactions [8]. Furthermore, the stimulation of the CD44–HER2complex by HA increases the growth of malignant cells and the presence of HA on the cancer cell surface may render the cells resistant to treatment [9].

Our study was designed to reveal the relationship between CD44 expression in human breast cancer and its role as indicator of tumor progression.

MATERIALS AND METHODS

This is a retrospective study that was carried out on eighty six cases diagnosed as invasive duct carcinoma retrieved from the Pathology Department, National Cancer Institute (NCI), Cairo University during the period from January 2009 to December 2010. Data regarding age, type of surgery and its date, tumor size, nodal metastasis, ER, PR, HER-2 and Ki-67 were collected. Clinical data and follow up were retrieved from the patients' files regarding date of diagnosis, patients' stage, neoadjuvant and adjuvant chemotherapy, radiotherapy, hormonal therapy, local recurrence and its date, distant metastasis and its date and site as well as last follow up date and status.

Histopathologic examination was done and tumors were graded according to Nottingham combined histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson grading system), also known as the Nottingham grading system [10]. Retrieval and examination of immunostained slides for ER, PR, HER2 and Ki-67 was done and cases were subsequently classified according to profile of validated immunohistochemical surrogate panel [11] into luminal A, luminal B, Her2/neu enriched and TNBC.

Paraffin embedded sections were made at 4 microns thickness and mounted on positive charged slides. Immunostaining for CD44 was done using Bench Mark XT Autostainer (Ventana) using CD44 mouse monoclonal antibody (clone MRQ-13, ready to use, Cell Marque). Membranous/cytoplasmic reaction was considered positive and the proportion of positive tumor cells [12] were detected as follows; 0 = 0% positive tumor cells, 1 = 1-10% positive tumor cells, 2 = 11-50% positive tumor cells, 3 = 51-75% positive tumor cells and 4 = 76-100% positive tumor cells. Expression of CD44 in the stromal cells (i.e., fibroblasts, myofibroblasts and endothelial cells) was graded according to the percentage of positive stromal cells [13] as follows: negative (0–5%); weak (6-25%); moderate (26–50%); strong (51–75%) or very strong (76-100%).

Patients were assigned a clinical stage according to the AJCC system [14]. Imaging data (include mammography, chest x-ray, head and neck, chest and abdominal CT scans as well as bone scan) were collected. Patients were followed since date of the surgical excision till date of the last visit to detect disease free survival (DFS). Dates of local recurrence, regional metastasis and distant metastasis were recorded.

For statistical analysis, numerical data were described in terms of means with standard deviation, medians and range, minimum and maximum for dispersion. Frequencies (number of cases) and percentages were used when appropriate. Chi-square test was used to compare qualitative variables. Disease free survival was determined using the Kaplan-Meier product limit method. Comparison between survival rates of different groups was determined using the log-rank test. Probability (P value) < 0.05 was considered to be significant. Disease-free survival (DFS) was defined as the duration from the date of primary surgery (complete remission) to the first local recurrence and/or distant metastasis or the last follow-up. Overall survival (OS) was the duration from the date of diagnosis to the time of breast cancer-related death or the last follow-up.

RESULTS

Clinico-Pathologic Parameters: The patients' age ranged from 28-89 years with mean age 50 years. Patients were staged as; T1 (16.3%), T2 (60.5%), T3 (17.4%) and T4 (5.8%). The majority of cases were grade II (91.9%) and the remaining cases were grade III. Twenty seven cases were associated with DCIS. Paget's disease of nipple was observed in four cases. Axillary lymph node metastases were detected in 66.3% of cases and accordingly cases were categorized as; N0 (33.7%), N1 (29.1%), N2 (17.4%) and N3 (19.8%). Consequently, cases were categorized as stage I (10.5%), stage II (44.7%) and stage III (44.7%). Half of cases were hormone positive and 23.3% of cases were HER2 positive. Cases were classified as luminal A (16.3%), luminal B (33.7%), HER2 enriched (23.3%) and triple negative (26.7%).

Seventy one cases (82.6%) were managed with radical surgeries; modified radical mastectomy, radical mastectomy and skin sparing mastectomy, while fifteen

cases (17.4%) underwent conservative surgery. Chemotherapy was given to seventy three cases (84.9%), while post-operative radiotherapy was given to 54 cases. Forty nine cases (57%) received hormonal therapy; TAM and Femara.

CD44 Expression: Twenty eight cases (32.6%) showed positive expression of CD44 in the tumor cells (Fig. 1); twelve cases were (1+), nine cases were (2+), four cases were (3+)and three cases were (4+).On the other hand, thirty cases (34.9%) showed positive immunostaining for CD44 in stromal cells (Fig. 2 A and B); four cases showed very strong stromal staining, two cases showed strong stromal staining, six cases showed moderate stromal staining and eighteen cases showed weak stromal staining.

Table 1 showed the association between CD44 expression and clinicopathologic variables. No statistically significant association was obtained between CD44 expression in tumor cells and each of age, tumor grade, nodal metastases, T stage, N stage, anatomic stage, hormonal status, Her-2 status and breast cancer subtypes. On the other hand, the prevalence of CD44 expression in stromal cells in cases with nodal metastasis was higher than its prevalence in node negative cases (40.4% versus 24.1%; respectively), but no significant statistical difference could be obtained. CD44 expression was observed to be rising in T1 through T3/T4 cases to be 21.4% in T1, 30.8% in T2 and 55% in T3/T4 cases, but significant statistical difference couldn't be obtained. There was also rising in CD44 expression among various N stages to be 24.1% in N0, 32% in N1, 46.7% in N2 and 47.1% in N3, but significant statistical difference could not be reached. CD44 expression in stromal cells was significantly associated with anatomic stage, being 22.9% among stage I/II cases in comparison to 50% among stage III cases.CD44 stromal expression among hormone positive cases was lower than it among hormone negative cases, 25.6% versus 44.2%; respectively with statistical difference close to significant level. There was strong association between CD44 expression in stromal cells and HER-2 positive cases; where 60% of HER-2 positive cases were CD44 positive in comparison to only 27.3% of HER-2 negative cases. There was difference between various breast cancer subtypes regarding CD44 expression in stromal cells; where it was least in luminal A; 21.4% and raised slightly in luminal B; 27.6%, then triple negative 30.4%, to be maximum in HER-2 enriched cases 60%, with statistical difference approached the significant level (p value 0.056).

Disease Free Survival (DFS) Analysis: The patients were followed for a median period of 36 months with estimated 3-year DFS 59.2%. At the end of follow up, 31cases developed distant metastasis. The main site of metastasis was bone (20 cases), followed by lung (15 cases), liver (10 cases), brain (5 cases) and lastly to non-regional lymph nodes (3 cases). Multiple metastatic sites were observed in seventeen patients. Local recurrence was reported in only two cases. The first one was a female patient, 50 years old, presented with luminal B subtype, staged as T2N1M0, with negative margins and recurrence was reported after two years of conservative surgery. The second one was a female patient, 76 years old, presented with HER-2 enriched subtype, staged as T2N2M0 with negative margins and recurrence was reported after one year of modified radical mastectomy.

Table 2 demonstrated that nodal metastasis, T stage, N stage, anatomic stage, hormonal status, HER-2 status and breast cancer subtypes are strong predictors for 3-year DFS. Regarding CD44 expression, it was found that CD44 expression in tumor cells is not significantly associated with DFS (p=0.800), in contrast to CD44 expression in stromal cells (Fig. 3), where CD44 negative cases achieved better DFS than CD44 positive cases (69.6% versus 40% respectively) (Fig. 4). On multivariate analysis, Her2 status and nodal stage (N0 versus N3) were significantly associated with 3-year DFS as shown in Table (3).

Overall Survival: Three-year overall survival (OS) was 68.1%. At the end of follow up, twenty seven patients died representing 31.4% of cases. Node negative breast cancer cases achieved better 3-year OS than node positive cases; 85.3% versus 59.1 %; respectively, with significant statistical difference (p value 0.009). T stage, N stage, anatomic stage, hormonal status, HER-2 status and breast cancer subtypes are significantly associated with 3-year OS (Table 2). CD44 expression in stromal cells was more predictive for 3-year OS than CD44 expression in tumor cells; where there was decline in 3 year OS from CD44 positive cases in relation to CD44 negative cases (56% versus 74.5%; respectively). This difference approached the significant level (p=0.057). This couldn't be obtained through analysis of association between CD44 expression in tumor cells and 3 year OS, where estimates for positive and negative cases were close, 63.9% versus 70.2%; respectively, with p value 0.800. On multivariate analysis (Table 3), only Her2 status and nodal stage (N0 versus N3), retained their significant association with 3-year OS.

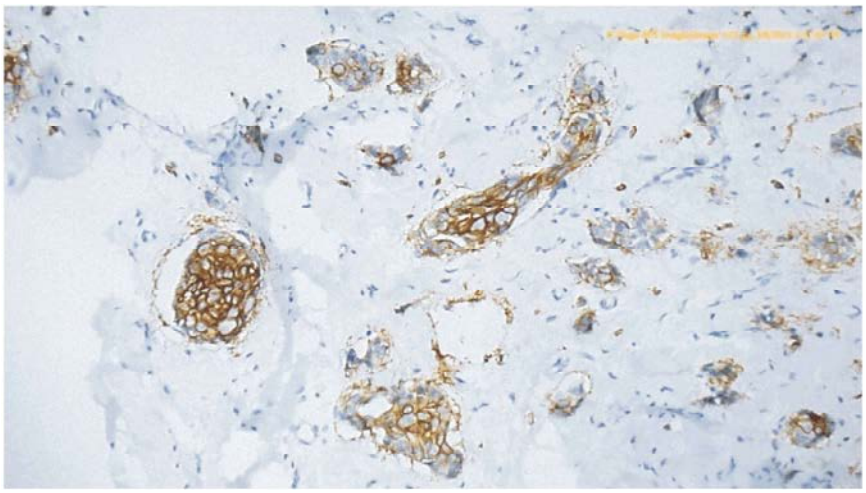

Fig. 1: A case of IDC, grade 2 in a female patient 54 years old, stage T2N2M0, classified as luminal B, showed CD44 membranous and cytoplasmic staining (3+) in the invasive tumor cells as well as tumor emboli (original magnification X200)

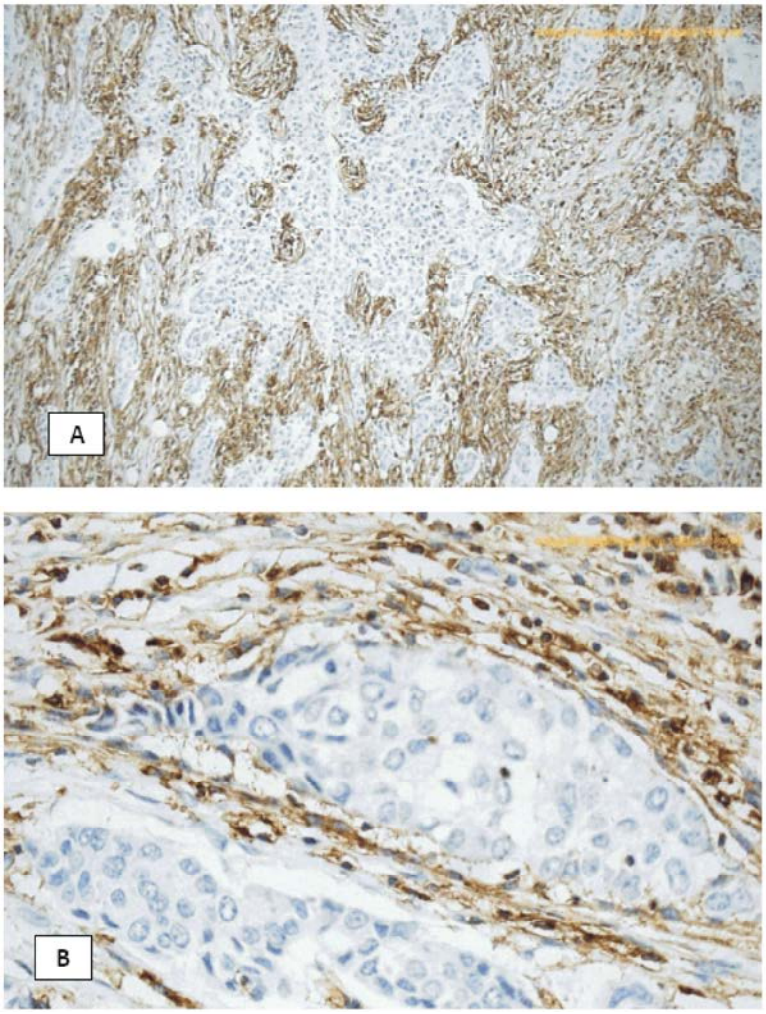

Fig. 2: A case of IDC, grade 2 in a female patient 68 years old, stage T2N2M0, classified as HER-2 enriched, showed CD44 stromal immunostaining (original magnification X100 (A)and X400(B)

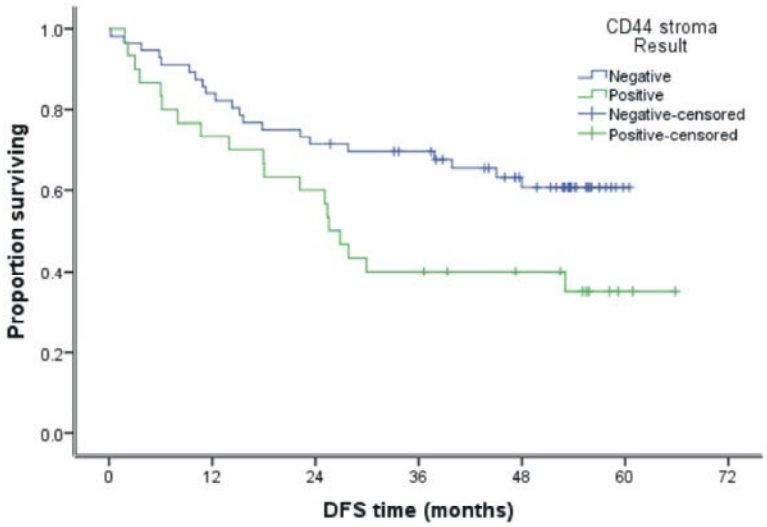

Fig. 3: DFS estimates in relation to CD44 stromal staining (*p* value0.023)

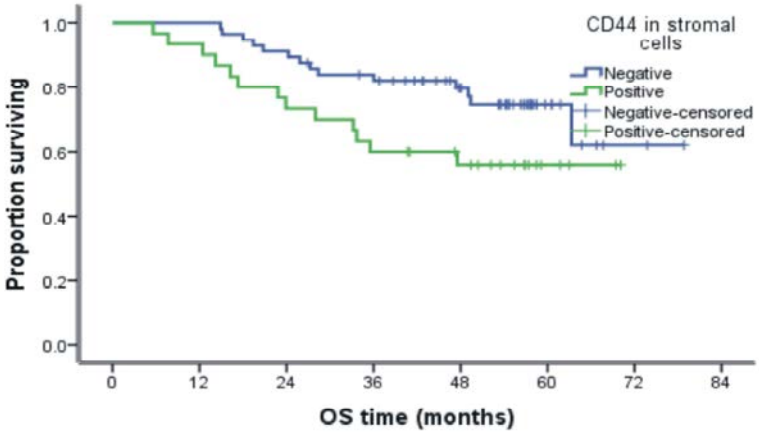

Fig. 4: OS estimates in relation to CD44 in stromal cells (p=0.057)

Table 1: The association between CD44 expression and clinicopathologic variables

	CD44 expression in tumor cells			CD44 expression in stromal cells		
Variable	Positive (no, %)	Negative (no, %)	p value	Positive (no, %)	Negative (no, %)	p value
Age						
<50	14 (37.8)	23 (62.2)	0.364	12 (32.4)	25 (67.6)	0.679
≥50	14 (28.6)	35 (71.4)		18 (36.7)	31 (63.3)	
Tumor Grade						
Grade II	26 (32.9)	53 (67.1)	1.000	26 (32.9)	53 (67.1)	0.232
Grade III	2 (28.6)	5 (71.4)		4 (57.1)	3 (42.9)	
Nodal Metastasis						
Present	18 (31.6)	39 (68.4)	0.811	23 (40.4)	34 (59.6)	0.159
Absent	10 (34.5)	19 (65.5)		7 (24.1)	22 (75.9)	
T Stage						
T1	4 (28.6)	10 (71.4)	0.163	3 (21.4)	11 (78.6)	0.079
T2	14 (26.9)	38 (73.1)		16 (30.8)	36 (69.2)	
T3/T4	10 (50)	10 (50)		11 (55)	9 (45)	
N Stage						
N0	10 (34.5)	19 (65.5)	0.864	7 (24.1)	22 (75.9)	0.309
N1	7 (28)	18 (72)		8 (32)	17 (68)	
N2	6 (40)	9 (60)		7 (46.7)	8 (53.3)	
N3	5 (29.4)	12 (70.6)		8 (47.1)	9 (52.9)	

Table 1: Continued

Anatomic Stage						
I/ II	12 (25)	36 (75)	0.204	11 (22.9)	37 (77.1)	0.009
III	16 (42.1)	22 (57.9)		19 (50)	19 (50)	
Hormonal Status						
Positive	15 (34.9)	28 (65.1)	0.645	11 (25.6)	32 (74.4)	0.070
Negative	13 (30.2)	30 (69.8)		19 (44.2)	24 (55.8)	
Her-2 Status						
Positive	7 (35)	13 (65)	0.790	12 (60)	8 (40)	0.007
Negative	21 (31.8)	45 (68.2)		18 (27.3)	48 (72.7)	
Breast Cancer Subtype						
Luminal A	5 (35.7)	9 (64.3)	0.895	3 (21.4)	11 (78.6)	0.056
Luminal B	10 (34.5)	19 (65.5)		8 (27.6)	21 (72.4)	
Her-2 enriched	7 (35)	13 (65)		12 (60)	8 (40)	
Triple Negative	6 (26.1)	17 (73.9)		7 (30.4)	16 (69.6)	

Table 2: Disease free and overall survival in relation to prognostic factors

Factors		No. of cases	No. of failures	3-year DFS	P-value	No. of cases	No. of failures	3-year survival	P-value
All cases		86	40	59.2		86	27	68.1	
Age groups	< 50 yrs	37	17	54.1	0.921	37	12	68.0	0.857
	≥ 50 yrs	49	23	63.1		49	15	68.3	
Tumor grade	grade 2	79	36	60.7	0.467	79	24	69.4	0.236
	grade 3	7	4	42.9		7	3	57.1	
Nodal metastasis	Present	57	32	49.1	0.006	57	23	59.1	0.009
	Absent	29	8	79.3		29	4	85.3	
T stage	T1	14	3	92.9	<0.001	14	0	100.0	<0.001
	T2	52	21	63.2		52	14	78.6	
	T3/T4	20	16	25.0		20	13	45.0	
N stage	N0	29	8	79.3	<0.001	29	4	85.3	<0.001
	N1	25	11	60.0		25	8	67.8	
	N2	15	6	60.0		15	3	78.8	
	N3	17	15	23.5		17	12	31.4	
Anatomic stage	I/II	48	13	80.3	<0.001	48	8	82.3	0.001
	III	38	27	36.8		38	19	50.4	
Hormonal status	Positive	43	14	72.1	0.010	43	8	83.3	0.002
	Negative	43	25	46.3		43	19	50.9	
Her2/neu	Positive	20	17	15.0	<0.001	20	14	28.6	<0.001
	Negative	66	23	72.6		66	13	80.6	
Breast Cancer Subtypes	Luminal A	14	5	64.3	<0.001	14	2	85.1	<0.001
	Luminal B	29	8	75.9		29	6	82.5	
	Her2/neu	20	17	15.0		20	14	28.6	
	Triple negative	23	8	73.7		23	5	77.5	
CD44 in tumor cells	Positive	28	14	57.1	0.800	28	10	63.9	0.561
	Negative	58	26	60.2		58	17	70.2	
CD44 in stromal cells	Positive	30	19	40.0	0.023	30	13	56.0	0.057
	Negative	56	21	69.6		56	14	74.5	

Table 3: Multivariate analysis for 3-year DFS and 3-year OS

	3-year DFS				3-year OS			
		95.0% CI for HR				95.0% CI for HR		
	HR	Lower	Upper	P-value	HR	Lower	Upper	P-value
Her2neu status	4.50	2.27	8.91	<0.001	4.25	2.12	8.50	<0.001
N0 vs N3	6.28	2.54	15.52	<0.001	0.38	0.18	0.79	0.010

DISCUSSION

CD44 is a transmembrane glycoprotein that participates in many cellular processes including regulation of cell division, survival, migration and adhesion [15] through the binding of its major ligand, hyaluronic acid (HA) and by acting as a cellular platform for growth factors. It can also act as a co-receptor to mediate signaling of the HER-2 family, possibly by organizing the assembly of functional complexes. CD44 also provides a link between the plasma membrane and the actin cytoskeleton, modulating cellular shape and motility [16].

Conflicting results are observed regarding the role of CD44 in breast cancer and its association with established histopathologic features. Some studies [17] demonstrated significant associations between CD44 expression and each of nodal metastasis and breast cancer subtypes but not with T stage, grade, hormonal status and HER-2 status. Others [13] found significant association between CD44 expression and each of tumor grade and ER status, but not with nodal status, HER-2 status and T stage. Moreover, one study [18] obtained significant association between CD44 and HER-2 status and hormonal status but not with patient's age, tumor grade, nodal status and T stage. Breast cancer subtypes were significantly associated with CD44 in only one study [19]. It is possible that the activation state of CD44 in carcinoma cells, like oligomerization is more important than the amount of the expression [20]. We also couldn't get any significant association between CD44 in tumor cells and any pathologic feature.

On the other hand, when we analyzed CD44 expression in stromal cells and we found significant association with HER-2 status, anatomic stage, DFS and borderline significance with OS. This was also reported by others [13]. Expression of CD44 in stromal cells was found to promote epithelial mesenchymal transition (EMT) by activating the PI3K/Akt signaling pathway. CD44 also has been reported to induce hyaluronan dependent mitogenic response in endothelial cells inducing and mediates metastatic adhesion of cancer cells released from the primary tumor [21].

Another point of view revealed that the expression of HA receptor CD44 was strongly associated with levels of hyaluronan synthases in stromal cells resulting in increased stromal expression of HA and CD44 which are important factors in inflammatory reaction [22] and the inflammatory component is important in the initiation and progression of cancer [23]. The CD44-HA axis likely recruit tumor inflammatory cells and modifies their functions to support malignant growth. This is reflected in our study by demonstration that stromal CD44 expression was associated with unfavorable outcome and worse prognostic factors in breast cancer.

MicroRNAs (miRNAs) are single-stranded RNAs of21–25 nucleotides in length, which have been found to modulate gene expression at the posttranslational level [24]. Overexpression of miR-21 influences cell proliferation, invasion, metastasis and chemoresistance in different cancer cells including breast cancer cells [25]. It was shown before that HA-CD44 interaction promotes miR-21production, contributing in upregulation of inhibitors of apoptosis proteins and the multidrug resistant protein (MDR1)/P-glycoprotein(P-gp) resulting in anti-apoptosis and chemotherapy resistance in breast tumor cells (MCF-7 cell line) [26].

CONCLUSIONS

We concluded that CD44 expression in stromal cells is more important in predicting the prognosis of breast carcinoma than its expression in tumor cells as it is associated with advanced stage, negative hormonal state, Her2 positive state, Her2 –enriched subtype and 2-year DFS. Experimental studies concerned with inhibition of the HA-CD44 link are suggested to create new potential target for treatment.

Conflict of Interest: The authors claimed that they have no conflict of interest.

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