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HER2-expressing breast tumors are associated with breast cancer stem-cell phenotype CD44$^+$/CD24$^-$

Tumores de mama que superexpressam HER2 estão associados ao fenótipo de células-tronco tumorais CD44$^+$/CD24$^-$

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ABSTRACT

Introduction: According to the cancer stem-cell theory, tumors originate from a small population of cancer stem cells, which lose the mechanism of self-regulation and begin to differentiate and proliferate indefinitely. The CD44$^+$/CD24$^-$ phenotype may be considered a stem-cell marker in breast cancer. **Objective:** To evaluate the correlation between CD44$^+$/CD24$^-$ phenotype and different molecular subtypes of breast cancer in invasive ductal carcinoma samples. **Methods:** The expression of CD44, CD44v6, and CD24 markers was investigated in 133 cases of invasive mammary carcinoma with immunohistochemistry. CD44$^+$/CD24$^-$ phenotype was identified and correlated with the molecular subtypes and classical prognostic factors such as age, histological grade, tumor size, and lymph node status. **Results:** Eighteen (14%) cases were positive for CD44$^+$/CD24$^-$ (CD44$^+$/CD24$^-$ or CD44v6+/CD24-) phenotype; among these, 11.1%, 27.8%, 38.9%, and 22.2% were luminal, luminal B-human epidermal growth factor receptor 2 (HER2), HER2, and triple-negative subtype, respectively. CD44$^+$/CD24$^-$ phenotype was more common in HER2 subgroup ($p = 0.0197$). **Conclusion:** CD44$^+$/CD24$^-$ phenotype was correlated with molecular subtypes of breast cancer. The highest expression of CD44$^+$/CD24$^-$ phenotype was reported in patients with HER2+ disease, a molecular subtype associated with more aggressive behavior and worse prognosis.

Key words: breast neoplasms; neoplastic stem cells; CD44 antigens; CD24 antigens; breast ductal carcinoma.

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed malignancy in women[1, 2] and the second most common cause of cancer death in females. It is recognized as a heterogeneous and phenotypically diverse disease characterized by great variability in the recurrence pattern and survival outcomes observed among different BC subtypes. The initial gene expression arrays based on the measurement of gene cluster identified distinct molecular subtypes such as luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal subtypes[3-6]. The luminal A subtype is enriched with genes similar to those found in luminal cells from the normal breast parenchyma and characterized by a high expression of hormone receptors and favorable outcome. The luminal B subgroup differs from luminal A in terms of high expression of proliferative genes, thereby presenting worse prognosis as compared with luminal A. HER2 and basal subtypes display poor prognosis in terms of overall survival and disease-free survival[4-6]. This analysis allowed the description of a tumor molecular portrait with different molecular subtypes harboring distinct prognosis[4, 5] and showing different responses to local and systemic therapy[7,8]. Molecular classification is based on the differences in the pattern of gene expression in tumors. However, genetic array testing may not always be feasible in clinical practice. Hence, immunohistochemical analysis has been applied to molecular classification (**Table 1**)[9-11]. The 12th St. Gallen International Breast Cancer Conference held in 2011[12] introduced the molecular subtypes in BC treatment and allowed approximation of the molecular classification with clinicopathological criteria based on the immunohistochemistry analysis. It was recently demonstrated that the discordance level

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TABLE 1 – Correlation between immunohistochemical analysis and gene expression for molecular classification

Molecular classification	Gene expression	Immunohistochemical expression
Luminal A	⇧ expression of luminal cell and hormone receptor genes	ER^+, PR^+, $HER2^-$ Luminal cytokeratin+ (CK8/18)
Luminal B	⇧ expression of hormone receptor and proliferative genes	ER^+, PR^+, $HER2^+$, high Ki67
HER2	⇩ expression of hormone receptor genes ⇧ expression of HER2 genes	ER^-, PR^-, $HER2^+$
Basal	⇩ expression of hormone receptor genes ⇧ expression of basal cell genes	ER^-, PR^-, $HER2^-$, basal cytokeratin+ (CK5/6, CK14, CK17)

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; CK: cytokeratin.

between molecular classification and clinicopathological criteria level may reach up to 30%[13].

In a cancer stem-cell model, the tumor originates from a small population of stem cells that lose regulation of the self-renewal process[14, 15]. The consequences are the differentiation and growth of these cells accompanied by genetic and epigenetic alterations. Resistance to therapy may be associated with this clonal evolution[16]. Some of these cells may be identified by cell surface markers, such as the presence of CD44 and absence of CD24 markers ($CD44^+/CD24^-$ phenotype)[17]. CD44 and CD24 were shown to promote[18] or inhibit[19] invasion and metastasis of BC cells. However, the phenotype $CD44^+/CD24^-$ was clearly associated with invasion, as $CD44^+/CD24^-$ subpopulation was more invasive than $CD44^+/CD24^-$ subpopulation of the same TMD-436 cell line[20]. $CD44^+/CD24^-$ phenotype was described as an indicator of stem cells in human breast tissue. These cells have been shown to survive cytotoxic therapy, leading to treatment failures and recurrences[21], besides being closely correlated with the invasive property and poor prognosis[22]. As cancer stem cells may be responsible for treatment resistance, these cell types are expected to be enriched in more aggressive subtypes, resulting in worse prognosis.

Here, we investigated the relationship between $CD44^+/CD24^-$ phenotype and molecular subtypes in BC using tissue microarray (TMA) technique. Furthermore, we examined the correlation of this phenotype with classical prognostic factors such as age, histological grade, tumor size, and lymph node status.

METHODS

Patients and specimens

Breast tumor samples of invasive ductal carcinoma from 133 patients who underwent surgery at the Breast Unit of Hospital Nossa Senhora das Graças (Curitiba, Brazil) between January 1998 and July 2009 were included in a non-consecutive manner. Patients that underwent mastectomy or breast-conserving surgery with sentinel node biopsy or axillary dissection and had a confirmed invasive ductal BC diagnosis were included in this study, while patients that underwent neoadjuvant chemotherapy or had insufficient paraffin-embedded tissue for the new analysis were excluded from the study.

Tissue microarray construction

Formalin-fixed paraffin-embedded (FFPE) blocks from multiple patients were visualized using TMA method to reduce the amount of reagents required for immunostaining of an entire array. Briefly, all FFPE tissue blocks were punched with a hollow needle and five tissue cores (3-mm wide) were removed. These samples were transferred into a positional encoded array in a paraffin block recipient that served as a new TMA. Tumor samples selected in this study were distributed in 12 TMAs (4-mm tissue cores), and sections from these TMAs were cut using a microtome, mounted on a microscope slide, and analyzed by immunohistochemistry. A normal breast tissue was included as an internal control in each TMA[23].

Immunohistochemical staining

Immunohistochemistry (IHC) for hormonal receptors as well as HER2 was performed during diagnosis using the streptavidin-biotin-peroxidase method. Samples were considered positive for estrogen and progesterone receptors when a weak, moderate, or strong nuclear staining was detected in more than 1% of the cells[24]. HER2 status was evaluated according to Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 guidelines[25], which classify tumor into four categories (0, 1+, 2+, 3+) based on the percentage of positive cells and intensity of membrane staining. Cases interpreted as 3+ were considered positive, whereas 2+ tumors were considered equivocal and referred to fluorescent *in situ* hybridization (FISH). Cases interpreted as 0 or 1+ were considered negative.

IHC staining for CD44, CD44v6, and CD24 was also performed with the streptavidin-biotin-peroxidase method in 12 TMA slices (**Table 2**). Staining for CD44 was conducted at the Pathology

TABLE 2 – Antibodies and antigen retrieval techniques

	Clone	Source	Dilution	Antigenic retrieval
Anti-CD44	Mouse monoclonal	Novocastra, UK	1:40	Immuno-retrieval: DAKO, pH 6, 20 min
Anti-CD44v6	Mouse monoclonal	Novocastra, UK	1:100	Immuno-retrieval: DAKO, pH 6, 20 min
Anti-CD24	Rabbit polyclonal	Abbiotec, USA	1:200	Immuno-retrieval: DAKO, pH 6, 20 min

Laboratory of A.C. Camargo Cancer Center (São Paulo), while that for CD24 and CD44v6 was carried out at the Experimental Pathology Laboratory of Pontifícia Universidade Católica do Paraná (PUCPR). The expression of CD44, CD44v6, and CD24 was considered positive when the membranous or cytoplasmic immunostaining was ≥ 5% (**Figure**)[26]. Samples detected positive for CD44 or CD44v6 and completely negative for CD24 were considered to display $CD44^+/CD24^-$ phenotype. The slides were simultaneously analyzed by a researcher and a pathologist using a multi-observer microscope, without any knowledge of the clinical case.

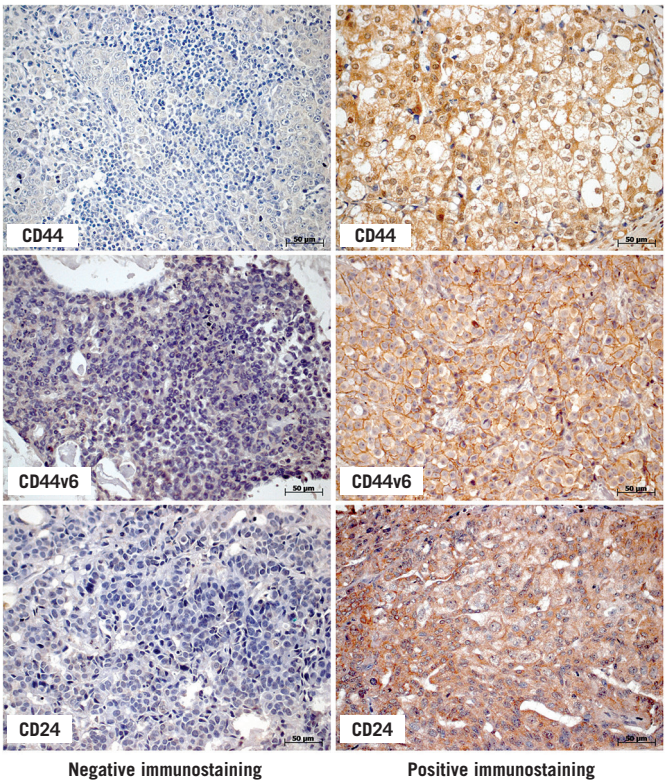

FIGURE – *IHC presentation of negative control (left panel) and positive (right panel) tissue for selected markers (40× magnification)*

IHC: immunohistochemistry.

Clinicopathological parameters

The age of patients was analyzed as a continuous variable by arithmetic mean and standard deviation. The histological grade was classified according to the Nottingham-Bloom-Richardson grading system[27]. Tumor size was reported according to tumor-node-metastasis (TNM) (7^{th} edition, 2009) classification of the American Joint Committee in Cancer[28]. Lymph node status was considered positive or negative according to the presence or absence of tumor cells in at least one lymph node. Invasive ductal carcinoma was defined according to the World Health Organization (WHO) Classification of Tumours of the Breast[29]. The sample was classified into four molecular subtypes based on clinicopathological criteria:

- luminal – estrogen receptor (ER)+ and/or progesterone receptor (PR)+ and HER2-;
- luminal B-HER2 – ER+ and/or PR+ and HER2+;
- HER2 – ER-, PR-, and HER2+;
- triple negative (TN) – ER-, PR-, and HER2-.

Statistical analysis

The Student's *t*-test for independent samples or analysis of variance (Anova) model was used to compare age between groups. Chi-square or Fisher's exact test was used to evaluate the association between qualitative variables. Logistic regression model was used for multiple comparisons of qualitative variables between more than two groups and to evaluate the subtypes based on $CD44^+/CD24^-$ phenotype (adjusting for age, histological grade, and tumor size). A value of $p < 0.05$ was considered statistically significant. Data were analyzed with the software Statistica v.8.0.

Ethical aspects

This study was approved by the Ethics Committee of PUCPR, registration number 5365.

RESULTS

Characteristics of the sample

The study comprised 133 cases:

- 42 cases of luminal (ER+ and/or PR+ and HER2-);
- 31 cases of luminal B-HER2 (ER+ and/or PR+ and HER2+);
- 21 cases of HER2 (ER-, PR-, and HER2+);
- 39 cases of TN (ER-, PR-, and HER2-).

The mean and median ages were 55.8 years (range 27-88 years, standard deviation 13.8) and 54 years, respectively. The sample distribution in relation to histological grade, tumor size, and lymph node is described in **Table 3**.

TABLE 3 – Distribution of the sample in relation to histological grade, tumor size, and lymph node

Number of cases (n = 133)		%
Histological grade		
I	18	13.5
II	64	48.1
III	51	38.3
Tumor size		
T1	56	42.1
T2	69	51.9
T3	8	6
T4	0	0
Lymph node		
Negative	75	56.4
Positive	58	43.6

Clinicopathological characteristics and molecular subtypes

Younger patients tended to harbor the TN subtype ($p = 0.028$). Of note, patients with TN BC had the lowest mean age. Individual comparison between groups showed that the average age of the TN cohort was significantly lower than that of the luminal phenotype ($p = 0.008$) and HER2+ ($p = 0.019$) cohort. Patients with TN BC and HER2+ disease had more aggressive conventional pathologic features. In comparison with luminal and luminal B-HER2 groups, the TN BC group had more grade III tumors ($p < 0.001$). Similar observation was reported for the HER2+ subgroup (**Table 4**).

Clinicopathological characteristics and CD44, CD44v6, and CD24 expression

The individual analysis of CD44, CD44v6, and CD24 resulted in the exclusion of four cases from CD24 group. In addition, four cases were excluded from the CD44v6 group; two lacked residual tumor on the slide and two histological sections had detached from the slide. One case was excluded from CD44 analysis because the histological section had detached from the slide. In 132 cases, 50% were positive for CD44 (66 cases). Out of 129 cases, 88 cases (68.2%) were positive for CD44v6, and 103 cases (79.8%) were positive for CD24. No significant association was observed between the expression of CD44, CD44v6, and CD24 and clinicopathological characteristics (**Table 5**).

Molecular subtypes, CD44+/CD24- phenotype, and CD44, CD44v6, and CD24 expression

Eighteen of the 129 (14%) samples were positive for CD44 and/or CD44v6 expression and negative for CD24 expression. Among patients with CD44+/CD24- phenotype, 13 (72%) were reported positive for CD44v6 expression and one (5.5%) was reported positive for CD44 expression; four (22.2%) patients showed expression of both markers. The evaluation of the expression of CD44+/CD24- phenotype in different BC subtypes revealed a significant difference between groups ($p = 0.02$); the highest frequency was reported for HER2+ group. Comparison of the subtypes in pairs showed that HER2+ group demonstrated a higher frequency of CD44+/CD24- phenotype as compared to luminal ($p = 0.008$) and TN ($p = 0.044$) group. Multivariate analysis revealed a higher frequency of CD44+/CD24- phenotype in HER2+ subtype than luminal ($p = 0.016$) and TN ($p = 0.050$) subtype (**Table 6**). No significant difference was observed in CD44 expression between groups

TABLE 4 – Clinicopathological characteristics of patients and comparison with molecular subtypes

Characteristic	Total (n = 133)	Luminal (n = 42)	Luminal B-HER2 (n = 31)	HER2+ (n = 21)	TN (n = 39)	p^*
Age (year)	55.8 ± 13.8	59.1 ± 15.2	54.8 ± 11.9	59.7 ± 13.8	51.1 ± 12.4	0.028
Histological grade						
I	18 (13.5)	9 (21.4)	7 (22.6)	1 (4.8)	1 (2.6)	
II	64 (48.1)	25 (59.5)	18 (58.1)	8 (38.1)	13 (33.3)	
III	51 (38.3)	8 (19)	6 (19.4)	12 (57.1)	25 (64.1)	< 0.001
Tumor size						
T1	56 (42.1)	19 (45.2)	14 (45.2)	10 (47.6)	13 (33.3)	
T2	69 (51.9)	22 (52.4)	16 (51.6)	9 (42.9)	22 (56.4)	
T3	8 (6)	1 (2.4)	1 (3.2)	2 (9.5)	4 (10.3)	0.618
Lymph node						
Negative	75 (56.4)	24 (57.1)	21 (67.7)	12 (57.1)	18 (46.2)	
Positive	58 (43.6)	18 (42.9)	10 (32.3)	9 (42.9)	21 (53.8)	0.348

**Chi-square test (categorical variables) or the model Anova for age; p < 0.05; *p value was calculated between the four molecular subtypes (luminal, luminal B-HER2, HER2, TN). HER2: human epidermal growth factor receptor 2; TN: triple negative; Anova: analysis of variance.*

TABLE 5 – Clinicopathological characteristics and CD44, CD44v6, and CD24 expression

Variable	CD44 (n = 132)			CD44V6 (n = 129)			CD24 (n = 129)		
	Negative (n = 66)	Positive (n = 66)	p^*	Negative (n = 41)	Positive (n = 88)	p^*	Negative (n = 26)	Positive (n = 103)	p^*
Age (years)	55.6 ± 14	56 ± 13.7	0.89	55.6 ± 14.7	56.1 ± 13.4	0.844	55.2 ± 14.1	56.2 ± 13.5	0.746
Histological grade									
I	11 (61.1)	7 (38.9)	0.264	6 (35.3)	11 (64.7)	0.378	4 (23.5)	13 (76.5)	0.494
II	27 (42.9)	36 (57.1)		23 (36.5)	40 (63.5)		10 (15.9)	53 (84.1)	
III	28 (54.9)	23 (45.1)		12 (24.5)	37 (75.5)		12 (24.5)	37 (75.5)	
Total	66 (100)	66 (100)		41 (100)	88 (100)		26 (100)	103 (100)	
Tumor size									
T1	24 (43.6)	31 (56.4)	0.012	18 (32.7)	37 (67.3)	0.593	12 (21.4)	44 (78.6)	0.247
T2	34 (49.3)	35 (50.7)		22 (32.8)	45 (67.2)		11 (16.7)	55 (83.3)	
T3	8 (100)	0 (0)**		1 (14.3)	6 (85.7)		3 (42.9)	4 (57.1)	
Total	66 (100)	66 (100)		41 (100)	88 (100)		26 (100)	103 (100)	
Lymph node status									
Negative	37 (50)	37 (50)	1	24 (33.3)	48 (66.7)	0.707	15 (20.8)	57 (79.2)	1
Positive	29 (50)	29 (50)		17 (29.8)	40 (70.2)		11 (19.3)	46 (80.7)	
Total	66 (100)	66 (100)		41 (100)	88 (100)		26 (100)	103 (100)	

**Chi-square test or Fisher's exact test (categorical variables) and Student's t-test for independent samples (age); p < 0.05; **a logistic regression model could not be adjusted for the two-to-two comparisons of tumor sizes because there were no cases of T3 tumor with CD44+ expression.*

TABLE 6 – Comparison between molecular subtypes and CD44 and CD24 phenotypes

Molecular subtype	$CD44^+/CD24^-$ (n = 129) Negative (n = 111) n (%)	Positive (n = 18) n (%)	p^*
Luminal	39 (95.1)	2 (4.9)	0.02
Luminal B-HER2	25 (83.3)	5 (16.7)	
HER2	14 (66.7)	7 (33.3)	
TN	33 (89.2)	4 (10.8)	
CD44v6 (n = 129)			
	Negative (n = 41) n (%)	Positive (n = 88) n (%)	p^*
Luminal	18 (45)	22 (55)	0.078
Luminal B-HER2	6 (20)	24 (80)	
HER2	4 (19)	17 (81)	
TN	13 (34.2)	25 (65.8)	
CD24 (n = 129)			
	Negative (n = 26) n (%)	Positive (n = 103) n (%)	p^*
Luminal	6 (14.6)	35 (85.4)	0.044
Luminal B-HER2	5 (16.7)	25 (83.3)	
HER2	9 (42.9)	12 (57.1)	
TN	6 (16.2)	31 (83.8)	
CD44 (n = 132)			
	Negative (n = 66) n (%)	Positive (n = 66) n (%)	p^*
Luminal	19 (46.3)	22 (53.7)	0.612
Luminal B-HER2	14 (45.2)	17 (54.8)	
HER2	10 (47.6)	11 (52.4)	
TN	23 (59)	16 (41)	

*HER2: human epidermal growth factor receptor 2; TN: triple negative; *Chi-square test; p < 0.05.*

(Table 6). Comparison of CD44v6 expression showed a tendency to be statistically significant (p = 0.078) between the groups and luminal B-HER2 and HER2+ subtypes had the highest expression (Table 6). The expression of CD24 was statistically (p = 0.044) correlated to BC subtypes, with HER2+ subtype showing the lowest proportion of positive cases (Table 6).

DISCUSSION

The most significant finding of this study was the association between $CD44^+/CD24^-$ phenotype and BC molecular subtypes (p = 0.02); HER2+ subtype is known for poor prognosis and showed the highest frequency. Such association has been reported for the first time. HER2 subtype has been previously related to another stem-cell marker, aldehyde dehydrogenase 1 (ALDH1)[30-33]. $CD44^+/CD24^-$ phenotype may be associated with BC subgroups having a more primitive cellular origin (such as basal and claudin-low subtypes). Although several studies have shown this association[32, 34-36], this statement remains debatable[37]. Honeth *et al*. (2011)[35] studied 240 tumors from a cohort of 445 patients surgically treated for stage II BC and found that the occurrence of $CD44^+/CD24^-$ phenotype was enriched in basal tumors, especially in hereditary BRCA1 tumors. Furthermore, the higher frequency of $CD44^+/CD24^-$ cells was associated with the basal subtype[32]. Ricardo *et al*. (2011)[36] analyzed 466 cases of invasive mammary carcinomas and eight BC cell lines and observed a statistically significant correlation

between CD44+/CD24- phenotype and molecular subtypes of BC; CD44+/CD24- phenotype was reported to be most frequent in basal tumors. In addition, Giatromanolaki *et al*. (2011)[34] described an association between CD44+/CD24- phenotype and TN tumors. On the other hand, Lu *et al*. (2011)[37] suggested that CD44+/CD24- cells may act as progenitor cells in transit and have no relation with molecular subtypes or clinical and pathological parameters of invasive ductal carcinoma.

To address the relationship between HER2 activation and stimulation of BC stem cells, Wang *et al*. (2010)[38] compared the phenotypic differences between two cell lineages derived from a common mammary epithelial stem cell with different HER2 expression. Flow cytometry demonstrated a higher frequency of CD44+/CD24- cells in the HER2+ cell line, suggesting that HER2 displays the ability to increase the proportion of CD44+/CD24- stem cells. Korkaya *et al*. (2008)[39] showed that HER2 overexpression increases the proportion of stem/progenitor cells, in normal mammary epithelial cells by using *in vitro* mammosphere assays, and the expression of stem-cell marker ALDH. The author also demonstrated that HER2 overexpression increases and HER2 blockade decreases the cancer stem-cell population in breast cancer cell lines and mouse xenografts.

The development of HER2-targeting agents such as trastuzumab has substantially changed the HER2-overexpressed tumors prognosis in the latest years, especially when used in the neoadjuvant setting[40]. Recent data have shown that probably the great results of these agents are related to its ability to target the breast cancer stem-cell population[41]. Traditionally these agents have been indicated only for tumors that overexpress HER2, but Paik *et al*. (2008)[42] reanalyzed clinical samples accrued to the National Surgical Adjuvant Breast and Bowel Project protocol B-31 (NSABP B31), for HER2 gene amplification, and identified 174 cases which were originally reported as HER2+ but did not actually have HER2 gene amplification. Surprisingly, these patients had the same benefit of trastuzumab as the patients that were HER2 positive. One of the hypotheses to explain this finding is that the HER2 pathway is activated in the cancer stem-cell populations of this group of patients[41]. To answer this question, a randomized prospective phase III trial, NSABP B47, is in progress to determine the benefits of adjuvant trastuzumab in patients that are not HER2 positive[41].

In the development of new anti-tumor drugs, targeting the cancer stem-cell populations can provide new strategies for breast cancer treatment. Bozepinib is one of the most active compound of new (RS)-4,1-benzoxazepin-purines against human breast and colon cancer cell lines. It shows very low inhibitory concentration 50 (IC50) values, in both breast and colon cancer cells, and induces cell death by apoptosis[43]. Ramirez *et al*. (2014)[44] further studied the mechanism of action of bozepinib and found that this agent is a selective inhibitor of HER2 positive breast cancer cells. Moreover, the authors showed that bozepinib inhibited both mammo- and colonosphere formation in subpopulations isolated by ALDH activity, and was able to induce apoptosis in the resistant ALDH+ subpopulations, presenting promising data about the action of this agent over cancer stem cells[44].

Cancer stem cells are commonly related to the mechanism of chemotherapy and radiotherapy resistance[16]. Tumors overexpressing stem-cell markers were expected to be associated with poor prognosis and clinicopathological characteristics of more aggressive behavior. However, the relationship between cancer stem-cell marker and prognosis remains controversial. The phenotype CD44+/CD24- was associated with more aggressive clinicopathological features and, consequently, poor prognosis[34, 45], although some contradictory studies have reported poor prognosis in BCs enriched with CD44-/CD24+[46]. It was recently demonstrated that cancer stem-cell markers may be identified in tumors with worst prognosis within the basal subtype characterized with a higher expression of CD44+/CD24- phenotype[47]. We failed to observe any relationship between CD44+/CD24- phenotype and the classic prognostic factors as age, histological grade, or lymph node status, as previously described in other studies[35, 37, 48, 49].

A major limitation of this study is that we performed simple immunostaining to characterize CD44+/CD24- population. Ricardo *et al*. (2011)[36] conducted a comparative study between simple immunostaining and double immunostaining with fluorescence microscopy using the same anti-CD44 and anti-CD24 in both TMA and whole tissue and found similar results with both techniques. Our study included IHC staining of CD44v6 to assess CD44+/CD24- phenotype. CD44 is encoded by a single gene located on 11p13 chromosome. The standard isoform (CD44s) is the most common, but approximately 20 variants of CD44 formed by alternative splicing have been described. CD44v6 is one of the most common and best studied CD44 variants known to play a role in cell migration and proliferation and, consequently, cancer progression and metastasis[50, 51]. In addition, it is associated with aggressive tumor behavior and poor prognosis[52]. Although our results have shown no statistical association between presence of CD44v6 and cancer subtypes, we observed a tendency of association between the expression of CD44v6 and the luminal B-HER2 and HER2+ subtypes. We also revealed higher rates of CD24 with cytoplasmic immunostaining (79.8%). A significant association was also demonstrated between CD24 negativity and BC molecular subtypes; HER2 was the most frequent subtype in this group, and the correlation

between BC subtypes and CD24 revealed the lowest expression of CD24 in HER2+ subtype.

CONCLUSION

Our results demonstrated an association between the $CD44^+/CD24^-$ phenotype and molecular subtypes, with the highest expression observed in HER2 subtype. Cancer stem cells are possibly responsible for the mechanism of resistance to BC treatment, owing to their ability to survive chemotherapy and radiotherapy. Identification of these cells through cancer stem-cell markers may possibly open new pathways for BC treatment.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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RESUMO

Introdução: *De acordo com a teoria das células-tronco tumorais, os tumores são originários de uma pequena população de células-tronco que perdem o mecanismo de autorregulação e começam a se diferenciar e proliferar indefinidamente. O fenótipo $CD44^+/CD24^-$ pode ser considerado um marcador de células-tronco tumorais no câncer de mama.* ***Objetivo:*** *Avaliar a correlação entre o fenótipo $CD44^+/CD24^-$ e os diferentes subtipos moleculares do câncer de mama em amostras de carcinoma ductal invasor.* ***Métodos:*** *A expressão dos marcadores CD44, CD44v6 e CD24 foi investigada em 133 casos de carcinoma mamário invasor por meio de imuno-histoquímica. O fenótipo $CD44^+/CD24^-$ foi identificado e correlacionado com os subtipos moleculares e os fatores prognósticos clássicos, como idade, grau histológico, tamanho do tumor e* status *do linfonodo.* ***Resultados:*** *Dezoito (14%) casos foram positivos para o fenótipo $CD44^+/CD24^-$ ($CD44^+/CD24^-$ ou CD44v6+/CD24-), sendo 11,1%, 27,8%, 38,9% e 22,2% dos subtipos luminal, luminal B-*human epidermal growth factor receptor 2 *(HER2), HER2 e triplo negativo, respectivamente. O fenótipo $CD44^+/CD24^-$ foi mais comum no subgrupo HER2 (*$p = 0,0197$*).* ***Conclusão:*** *O fenótipo $CD44^+/CD24^-$ foi correlacionado com os subtipos moleculares do câncer de mama. A maior expressão do fenótipo $CD44^+/CD24^-$ foi encontrada em pacientes com doença HER2+, subtipo molecular associado a um comportamento mais agressivo e a um pior prognóstico.*

Unitermos: *neoplasias da mama; células-tronco neoplásicas; antígenos CD44; antígenos CD24; carcinoma ductal de mama.*

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