RESEARCH ARTICLE

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Immunohistochemical Expression of Autophagy-Related Marker *(LC3B)* and Stem Cell Marker *(CD44)* in Molecular Subtypes of Breast Cancer

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Abstract

Background: Breast cancer (BC) is among the most prevalent aggressive type of malignancy affecting females worldwide. Despite the advance in early detection and management of BC; recurrence, metastasis and mortality remains high. This may be attributed to heterogeneity of BC which explained by the presence of breast cancer stem cells (BCSCs). BCSCs is characterized by their ability of self-renewal, unlimited proliferation and their differentiation potential. BCSCs maintain their activity through process of autophagy. Autophagy is a catabolic pathway important for maintenance of cellular hemostasis in response to different stressful conditions. Autophagy allows BCSCs to adapt to different stressful conditions. So, it protects BCSCs from cytotoxic effects of anti-cancer therapy and anticancer resistance. **Methods:** Formalin-fixed paraffin embedded fifty specimens of Invasive duct carcinoma of no special type(IDC/NST) of breast was selected and immunostained with stem cell marker *CD44* and autophagy related marker *LC3B* antibodies. Correlation with different clinicopathological, histopathological characteristics and molecular subtypes of studied specimens were evaluated. **Results:** Both *CD44* and *LC3B* expression were significantly associated with lymph nodal metastasis (p =0.001 and 0.010 respectively), advanced pathological stage (p= 0.045 and 0.004 respectively) and with triple negative molecular subtype of BC (p=0.044 and 0.048 respectively). Statistically positive correlation was also found between both tumor markers expression. **Conclusion:** Results of this study suggests that *CD44* and *LC3B* expression play a role in the clinical behavior and progression of different molecular subtypes of BC.

Keywords: *CD44- LC3B-* breast cancer- IHC

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Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer related death in women worldwide [1]. It accounts for 23% of all malignancies with the infiltrating duct carcinoma considered the most frequent histological subtype [2]. Based on gene expression profiles, BC has been classified into 5 subgroups: luminal A, luminal B, Her2/neu amplified, basal like and normal like. Each group has distinct biological features and clinical outcome, suggesting that BC progresses through different molecular pathways among patients [3].

The heterogeneity in BC subtypes was suggested by some investigators to be a function of cancer stem cells (CSCs) [4], based on the common phenotypes between them and BC cells. This makes some authors to hypothesize that CSCs can be incorporated in the molecular staging of BC [5]. CSCs are defined by the expression of specific cell surface markers that can be used to distinguish them from other tumor or normal cells. *CD44* is one of these markers [6]. *CD44* is a member of the adhesion molecule families and a cell-surface trans-membrane protein distinguishes breast cancer stem cells (BCSCs) from breast Non- CSCs [6, 7]. It is closely associated with cancer metastasis, chemotherapy resistance and poor prognosis [8, 9].

CSCs are characterized by their ability of self-renewal, unlimited proliferation and their differentiation potential, thereby tumor therapy resistance and metastasis [8]. One of the pivotal processes that have been strongly associated to CSCs maintenance and aggressiveness is autophagy.

Autophagy, a conserved catabolic pathway, is crucial for the preservation of cell homeostasis during nutrient and oxygen deprivation [10]. Autophagy plays a dual role in cancer development; it can both promote and suppress cancer progression and metastasis. These opposite functions were interpreted as autophagy being a double-edged sword in cancer; that prevents tumor initiation but favors the progression of established tumors, this challenged researchers to further explore its impact on oncogenesis and tumor progression [11].

The membrane-bound microtubule-associated protein

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chain 3 (MAP- LC3) is one of the most specific biomarkers of autophagy. In mammals, LC3 is expressed in 3 isoforms: A, B, and C. The B isoform, *LC3B*, has broad tissue specificities and is widely used in autophagy-related studies [12]. The role of *LC3B* in different types of cancer including BC remains controversial. Flynnand Schiemann [11] and Tang et al. [13] reported the common expression of *LC3B* in various malignancies and its relation to cancer progression and worse outcome, while others recorded its relation to suppression of tumorgenesis [11, 13].

The relationship between autophagy and CSCs in BC remains unclear; some studies demonstrated that, BCSCs that exhibit high percentage of autophagic activity having poor prognosis [14]. While other studies showing opposite results with *LC3B* autophagic marker found to suppress function of BCSCs [15].

Therefore, autophagy could represent a promising target for counteracting CSCs aggressiveness that it would be crucial to carefully assess the dependence/sensitivity of each specific type of cancer to autophagy, as well as the impact of autophagy modulation on selected cancer therapies [16]. To date, our knowledge about the effect of autophagy on BCSCs is limited. Here we hypothesize that autophagy may have an impaction on BCSCs in different molecular subtypes of BC. To assess the credibility of our hypothesis we decide to study the expression of *LC3B* and *CD44* in different molecular subgroups of BC via immunohistochemistry.

Materials and Methods

Specimens

This study was conducted retrospectively on 50 specimens of IDC/NST of breast. Specimens were selected from the archives of Surgical Pathology Laboratories, Assuit University Hospital, Faculty of Medicine and South Egypt Cancer Institute, Assuit University.

The available clinicopathologic data were obtained from hospital medical records of patients and all the patients received treatment according to their stages and luminal subtypes according to national guidelines of breast cancer patient protocol. A representative hematoxylin and eosin stained slides were re-examined for each specimen for detailed histopathological features including histologic type, grade (Nottingham modification of the Scarff Bloom Richardson grading, 1998), tumor infiltrating lymphocytes, the presence or absence of lymohovascular emboli, the presence or absence of perineural invasion and tumor stage by using TNM classification published by the American Joint Committee on Cancer/Union for International Cancer Control (8th edition, 2018) [17].

According to the molecular classification, the specimens were divided into 2 groups, 25 specimens of ER +ve (both luminal A and B) and 25 specimens of ER-ve (HER2/neu +v and triple negative) BC. All procedures performed in the current study were approved by national research ethics committee (reference number17100680 on18/3/2019) in accordance with the 1964 Helsinki declaration and its later amendments.

Immunohistochemical staining

Immunohistochemical staining was performed using the avidin -biotin immunoperoxidase methods. Tissue sections of 4-µm thick of formalin-fixed paraffin-embedded specimens were taken from tissue blocks. Sections were dewaxed and then rehydrated through descending graded ethanol series, down to distilled water. To block the endogenous peroxidase, the rehydrated sections were treated with Peroxidase Blocking Reagent (6%hydrogen peroxide) for 10 min. For epitope retrieval, sections were microwaved in Tris EDTA solution, pH 9 for 1 hour. Sections were incubated with the primary antibodies for one hour.

The antibody used for *CD44* immunostaining was primary *CD44 /H-CAM* Rabbit Unconjugated polyclonal antibody (Catalog #PAS-29590, thermo scientific at dilution 1/200). As regard *LC3B* immunostaining, it was carried out using *LC3B* polyclonal unconjugated Rabbit Primary Antibody (catalog # PA1-16931, thermo scientific at dilution 1/300).

Secondary staining kits were used according to the manufacturer's instructions using Ultra Tek Anti-Polyvalent Biotinylated Antibody. Counter staining was done with hematoxylin and examined by light microscopy

Sections of appendix and pancreas were used as a positive control for *CD44* and *LC3B* marker respectively.

Immunohistochemical evaluation

All stained specimens were independently viewed and scored by two pathologists without disclosing clinical data of these patients.

CD44 scoring method

The immunostaining score for *CD44* was calculated based on the proportion of stained tumor cells: 0-10% as negative, 11-25% as weakly positive, 26-50% as moderately positive, and 51-100% as strongly positive. Patients with negative and weakly positive expression were combined as the lower expression group, and patients with moderately positive and strongly positive expression were combined as the higher expression group [18].

LC3B scoring method

The stained sections were scored taking into consideration the intensity of staining and the percentage of stained cells within each tissue section. The intensity of staining was scored as (0, no staining; 1, faint staining; 2, moderate staining; and 3, strong staining.) and the percentage of positively stained cells was also scored as (0, no staining; 1, 1–25 % positive cells; 2, 26–50 % positive cells; 3, 51–75 % positive cells; and 4, 76–100 % positive cells). The sum of the staining intensity and extent scores was used as the final score for *LC3B* (0–7). Patients showed final staining scores of 6–7 were classified as *LC3B* high expression and the remainder as *LC3B* low expression [19]. All procedures performed in the current study were approved by IRB and/or national research ethics committee in accordance with the 1964 Helsinki declaration and its later amendments.

Informed consent was obtained from all individual participants included in the study.

Statistical analysis

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Data were statistically described in frequencies (number of cases) and relative frequencies (percentages). For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P-value is always 2 tailed set significant at 0.05 levels. Correlation between *CD44* and *LC3B* was done by using Spearman's rho correlation test.

Results

Clinicopathological, histopathological characteristics and molecular subtypes of the studied specimens

The patient's clinicopathological, histopathological characteristics and molecular subtypes were summarized in (Table 1). Briefly all studied cases were IDC/NST of BC. They all were grade II. The age range was 27-65 years (mean, 50±10 years), with twenty six [52%] of cases were < 50 years old. As regard the molecular subtype, fourteen specimens [28%] were luminal A subtype, eleven specimens [22%] were luminal B subtype, eleven specimens [22%] were Her2neu overexpressing subtype and fourteen specimens [28%] were triple negative subtype.

Immunohistological findings of the studied specimens
Expression of CD44 and LC3B

CD44 was expressed in IDC cells with cytoplasmic and membranous staining pattern. Forty-six out of fifty specimens [46/50] were positive for *CD44* marker. The specimens were divided into two subgroups, with twenty-five specimens [50%] showed high expression and twenty-five specimens [50%] showed low expression (Figure 1).

As regard *LC3B* expression, it was expressed in IDC cells with cytoplasmic staining pattern. All fifty specimens showed positivity for *LC3B* in various proportion and different intensity. Finally, they were divided into two

Table 1. The Clinicopathological, Histopathological Characteristics and Molecular Subtypes of All Studied Specimens (n=50)

Variable		N	(%)
Age (years)	< 50	26	-52
	≥ 50	24	-48
Tumor size (cm)	≤ 5 cm	30	-60
	> 5 cm	20	-40
LN metastasis	Negative	15	-30
	N1	15	-30
	N2	11	-22
	N3	9	-18
Distant metastasis	Present	5	-10
	Absent	45	-90
Pathologic stage	Stage 1	1	-2
	Stage 2	20	-40
	Stage 3	24	-48
	Stage 4	5	-10
Histologic type	IDC	50	-100
Grade	Grade 2	50	-100
LVI	Present	37	-74
	Absent	13	-26
PNI	Present	8	-16
	Present	42	-84
DCIS	Present	31	-62
	Absent	19	-38
Tumor infiltrating Lymphocytes (TILS)	Mild	29	-58
	Moderate	18	-36
	Brisk	3	-6
Molecular subtype	Luminal A	14	-28
	Luminal B	11	-22
	Triple negative	14	-28
	Her2neu overexpression	11	-22

N, number of cases; LVI, lymphovascular invasion; PNI, perineural invasion; Her2, human epidermal growth factor receptor 2; Qualitative data are preQualitative data are presented in the form of number (percentage).

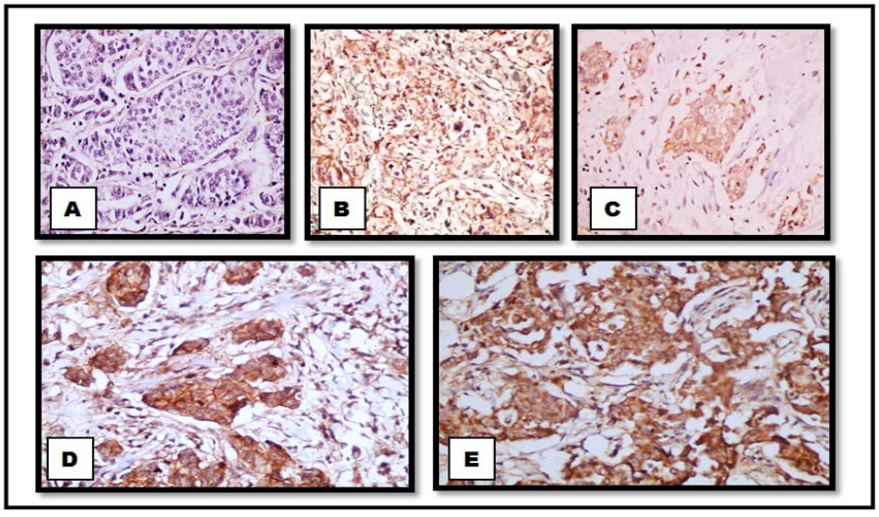

Figure 1. *CD44* Expression in IDC/NST of Breast. A, Negative expression of *CD44* (x400); B, Low expression of *CD44* (membranous staining) (x400); C, Low expression of *CD44* (cytoplasmic staining) (x400); D, High expression of *CD44* (membranous staining) (x400); E, High expression of *CD44* (cytoplasmic staining) (x400).

Table 2. Relationship between *CD44* & *LC3B* Expression and Clinicopathological Parameters of the Studied Specimens (n=50)

Variable	CD44 low expression		CD44 high expression		P value	LC3B low expression		LC3B high expression		P value
	(n=25)	(%)	(n=25)	(%)		(n=11)	(%)	(n=39)	(%)	
Age (years)										
< 50	13	-52	13	-52	1	4	-36.4	22	-56.4	0.24
≥ 50	12	-48	12	-48		7	-63.6	17	-43.6	
DCIS										
Present	16	-64	15	-60	0.771	8	-72.7	23	-59	0.498
Absent	9	-36	10	-40		3	-23.3	16	-41	
TILS										
Mild	13	-52	16	-64		5	-45.5	24	-61.5	
Moderate	10	-40	8	-32	0.66	6	-54.5	12	-30.8	0.369
Brisk	2	-8	1	-4		0	0	3	-7.7	
LVI										
Present	16	-64	21	-84	0.107	6	-54.5	31	-79.5	0.126
Absent	9	-36	4	-16		5	-45.5	8	-20.5	
PNI										
Present	4	-16	4	-16	1	2	-18.2	6	-15.4	1
Absent	21	-84	21	-84		9	-81.8	33	-84.6	
Tumor size										
≤ 5 cm	13	-52	17	-68	0.248	6	-54.5	24	-61.5	0.736
> 5 cm	12	-48	8	-32		5	-45.5	15	-38.5	
LN metastasis										
N0	13	-52	2	-8	0.001*	7	-63.6	8	-20.5	0.010*
N1/N2/N3	12	-48	23	-92		4	-36.4	31	-79.5	
Distant metastasis										
Present	1	-4	4	-16	0.349	1	-9.1	4	-10.3	1
Absent	24	-96	21	-84		10	-90.9	35	-89.7	
Pathologic stage										
Stage 1,2	14	-56	7	-28	0.045*	9	-81.8	12	-30.8	0.004*
Stage 3,4	11	-44	18	-72		2	-18.2	27	-69.2	

Qualitative data are presented in the form of number (percentage), * Significance defined by $p < 0.05$.

groups; low and high expression groups, with eleven specimens [22%] showed low *LC3B* expression and thirty-nine specimens [78%] showed high *LC3B* expression (Figure 2).

Relationship between CD44 & LC3B expression and clinicopathological characteristics

Statistically significant positive association was detected between high expression of both *CD44* & *LC3B* and lymph node metastasis (p value = 0.001 and 0.010 respectively) (Table 2). Statistically significant positive

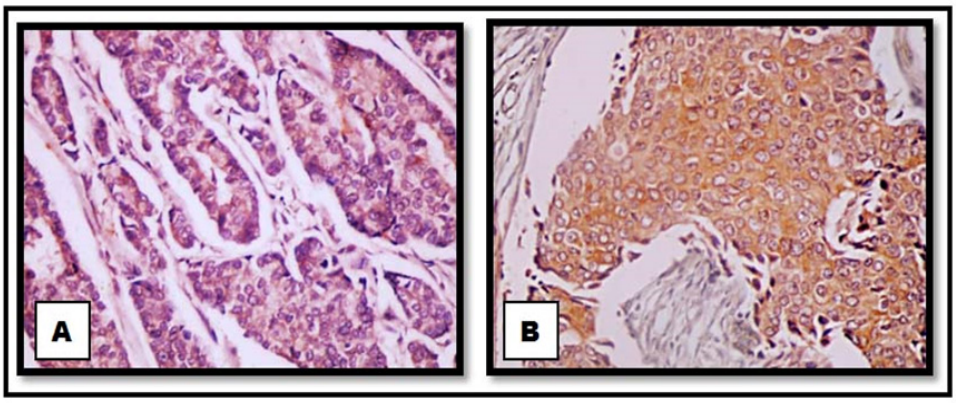

Figure 2. *LC3B* Expression in IDC/NST of Breast. A, Low expression of *LC3B* (cytoplasmic staining) (x400); B, High expression of *LC3B* (cytoplasmic staining) (x400).

Table 3. Relationship between *CD44& LC3B* Expression and Molecular Subtypes of the Studied Specimens (n=50)

Variable	CD44 low expression		CD44 high expression		P value	LC3B low expression		LC3B high expression		P value
	(n=25)	(%)	(n=25)	(%)		(n=11)	(%)	(n=39)	(%)	
Molecular subtype					0.044*					0.048*
Luminal A	9	-36	5	-20		5	-45.5	9	-23.1	
Luminal B	8	-32	3	-12		2	-18.2	9	-23.1	
Her2neu –OE	5	-20	6	-24		4	-36.4	7	-17.9	
Triple negative	3	-12	11	-44		0	0	14	-35.9	

Her2neu –OE, Her2neu overexpression; Qualitative data are presented in the form of number (percentage), * Significance defined by $p < 0.05$.

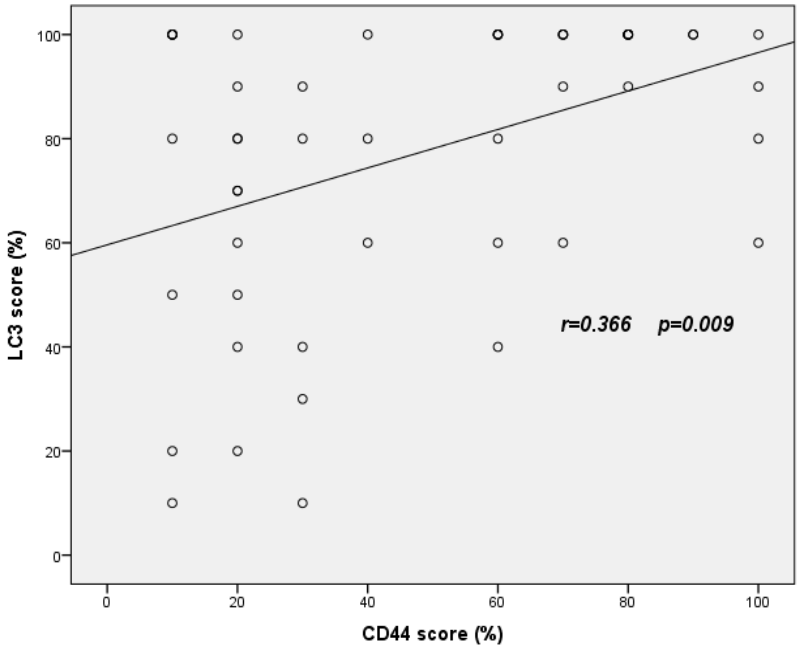

Figure 3. Scatter Plot Graph Showing the Correlation between the Percentage of CD44 and LC3B Markers. Significant positive moderate correlation was found between the percentage of CD44 and LC3B markers (r=0.366 and p=0.009).

association was also detected between *CD44 & LC3B* expression and pathologic stages. Patients with advanced stage (stage 3, 4) had high *CD44 & LC3B* expression (p value=0.045 and 0.004 respectively) (Table 2). There was no statistically significant relationship between both *CD44 & LC3B* expression and other clinicopathologic parameters such as age of patients, site and size of tumor, LVI, PNI, and DCIS (Table 2).

Relationship between CD44 & LC3B expression and molecular subtypes of studied specimens

Statistically significant positive association was detected between *CD44 & LC3B* expression and different molecular subtypes. Patients with triple negative subtype had higher *CD44 & LC3B* expression (44% and 35.9% respectively) than her2neu (24% and 17.9% respectively) and luminal subtypes (luminal A (20% and 23.1%respectively) & luminal B (12% and23.9% respectively) (p value=0.044 and 0.048 respectively) (Table 3).

Correlation between CD44 and LC3B tumor markers expression of the studied specimens

Significant positive moderate correlation was found between the percentage of *CD44* and *LC3B* markers (r=0.366 and p=0.009) (Figure 3).

Discussion

Breast cancer is a complex disease with large heterogeneity, leading to highly variable clinical behavior and response to therapy. The mechanisms resulting in this heterogeneity in BC are not well-understood with one possible explanation for the tumor heterogeneity is the presence of BCSCs [20]. The implication of the CSC model in BC has been suggested to account for potential differences in drug sensitivity, individual risk of recurrence and metastasis. So understanding of CSCs model can improve our ability to regulate target therapy [20].

Anticancer resistance of CSCs, cancer recurrence and metastasis are attributed to several factors including process known as autophagy [19, 21]. MAP1 LC3 is identified as marker of autophagy which played an important role in the development of BC [19]. Relationship between *CD44* expression and different clinicopatho-logical factors

In this study forty-six out of fifty specimens [92%] showed positive *CD44* expression. These results agree

with results of Farida and Yuliantini [22], they found that *CD44* expression was positive in [88.6%] of examined BC cases [22]. In this research high *CD44* expression was significantly associated with presence of axillary lymph nodal metastasis. This finding is in agreement with results of other previous studies such as Wei et al. [23], Rustamadji et al. [24], Tsang et al. [25].

The relation between high *CD44* expression and LNs metastasis can be explained as the following: *CD44* is a class I transmembrane glycoprotein that serves as the primary receptor for hyalouronic acid (HA) and binds other extra-cellular matrix components, such as collagen, laminin, and fibronectin. This protein has been shown to promote growth, invasion, and metastatic dissemination of BC cells [20]. However, other studies disagree with our results; they found no significant associations between *CD44* expression and LNs status in studied BC cases [18, 26, 27].

Pathologic stage is the most useful predictor of BC behavior. In the current study, statistically significant positive association was detected between high *CD44* expression and patients with advanced stage (stage 3, 4). This finding is in accordance with results of Hassn Mesrati et al. [28], Roosta et al. [18]. Lymph nodal status is one of the important determinants of pathologic stage. Since, *CD44* facilitates LN metastasis; its high expression is associated with advanced stage.

Relationship between CD44 expression and different molecular subtypes of BC

We found statistically significant association between *CD44* expression and different molecular subtypes of BC. More frequent CSC phenotypes, (higher *CD44* expression), were found in triple negative tumors [44% of specimens].

This finding is in agreement with results of previous studies done by Louhichi et al. [20]. They found that *CD44+* subpopulation was much higher in basal like BC than non-basal like subtype [20]. *CD44+* cells showed high capacity of proliferation, migration, invasion and tumorigenesis, so providing a highly hydrated environment that favors cancer cell progression and invasion (important features of basal like BC) [29].

In contrast, Chang et al. [15], Faridaand Yuliantini [22], de Beca et al. [30] found no significant association between stem cell marker *CD44* and different molecular subtypes of BC [15, 22, 30].

Relationship between LC3B expression and different clinicopatholo-gical parameters

The results of this work showed that level of *LC3B* expression was up-regulated during later stages of BC, where patients with advanced stage (stage 3, 4) showed high *LC3B* expression [69.2%] versus patients with low stage [18.2%]. These results are consistent with results of previous study by Zhao et al. [19].

Autophagy serves as a protective mechanism to cancer cells against stress conditions that affect cancer cells especially during later stages of the tumor development (where limited oxygen and nutrient supplies). It provides amino acids, nucleotides and lipid for ATP production and molecular synthesis of cancer cells under these stressful conditions, so promotes viability of the tumor cells [19]. On contrary, Mustafa et al. [31] found no significant association between *LC3B* expression and different pathologic stages of BC [31].

As regard lymph nodal status, a significant positive association between high *LC3B* expression [79.5%] and presence lymph node metastasis was detected. This finding agrees with results of Zhao et al. [19], and disagrees with Mustafa et al. [31]. These results are partially explained by ability of autophagy to help tumor cells to survive, proliferate and disseminate to regional lymph nodes and secondary sites [32]. In addition, Autophagy was shown to be induced during extracellular matrix (ECM) detachment and protects detached tumor cells from detachment-induced cell death, so tumor cells can survive and metastasize [33, 32].

Relationship between LC3B expression and different molecular subtypes of BC

Regarding molecular subtypes of BC, we found significant association between *LC3B* expression and different molecular subtypes of BC. Patients with triple negative subtype had higher *LC3B* expression [35.9%] than the remaining BC subtypes. Results of the current study agree with results of Choi et al. [34] and disagree with results of Mustafa et al. [31].

This finding can be explained as a following: TNBC cells showed higher level of hypoxia (reflected microscopically by central necrosis and high mitotic activity) than other subtypes of BC. Hypoxia is main stimulus of induction of autophagy and autophagy related markers so TNBC subtype showed higher *LC3B* expression than other subtypes [34]. This indicated that autophagy might promote viability and progression of TNBC cells [19].

Correlation between CSC marker CD44 and autophagy related marker LC3B

Autophagy allows BCSCs to adapt to different stressful conditions and to maintain their activity [35]. So, it protects BCSCs from cytotoxic effects of anti-cancer therapy [10].

Results of this study found significant positive moderate correlation between the percentage of *CD44* and *LC3B* markers in studied specimens. This results coincides with study on pancreatic cancer observed that pancreatic CSCs exhibited elevated autophagy in both clinical specimens and cell lines [36] and with another study was done by Wong et al. [37] to elucidate the interplay of autophagy and CSCs in hepatocellular carcinoma [37]. There are several medications that can exert effects on autophagy, which are also critical in BC therapy [38]. Results of this study suggest that the majority of inhibitors of autophagy may play an important role in both BC and BCSC improving the outcome in BC patients.

In conclusion; results of this study revealed that both stem cell marker *CD44* and autophagy related marker *LC3B* expression are significantly associated with lymph nodal metastasis, advanced pathological stage and with triple negative molecular subtype of BC. Statistically

positive correlation was also found between both tumor markers. There were no relationships between *CD44* & *LC3B* expression and other clinicopathologic parameters such as age of patients, site, and size of tumor, LVI, PNI, TILs, and DCIS were found.

Author Contribution Statement

Conceptualization: HEB; Data curation: HEB; Formal analysis: HEB, SH; Investigation: HEB, SH, MAH; Methodology: HEB; Project administration: HEB, SH.MAH; Resources: HEB, SH; Supervision: HEB.MAH; Validation: HEB; Visualization: HEB; Writing – original draft: HEB, SH; Writing – review & editing: HEB; Approval of final manuscript: all authors

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Conflict of interest

The authors declare that they have no conflict of interest exist.

Ethical issue

All procedures performed in the current study were approved by national research ethics committee (reference number17100680 on18/3/2019) in accordance with the 1964 Helsinki declaration and its later amendments.

Data availability

All data generated or analyzed during this study are included in this published article.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60(5):277-300. https://doi.org/10.3322/caac.20073.
3. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5(10):2929-43.
4. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell. 2010;140(1):62-73.
5. Malhotra GK, Zhao X, Band H, Band V. Histological, molecular and functional subtypes of breast cancers. Cancer Biol Ther. 2010;10(10):955-60. https://doi.org/10.4161/cbt.10.10.13879.
6. Sin WC, Lim CL. Breast cancer stem cells-from origins to targeted therapy. Stem Cell Investig. 2017;4:96. https://doi.org/10.21037/sci.2017.11.03.
7. Kamel M, Shouman S, El-Merzebany M, Kilic G, Veenstra T, Saeed M, et al. Effect of tumour necrosis factor-alpha on estrogen metabolic pathways in breast cancer cells. J Cancer. 2012;3:310-21. https://doi.org/10.7150/jca.4584.
8. Phi LTH, Sari IN, Yang Y-G, Lee S-H, Jun N, Kim KS, et al. Cancer stem cells (cscs) in drug resistance and their therapeutic implications in cancer treatment. Stem Cells Int. 2018;18(5):20-4.
9. Senbanjo LT, Chellaiah MA. *CD44*: A multifunctional cell surface adhesion receptor is a regulator of progression and metastasis of cancer cells. Front Cell Dev Biol. 2017;5:18.
10. Han Y, Fan S, Qin T, Yang J, Sun Y, Lu Y, et al. Role of autophagy in breast cancer and breast cancer stem cells (review). Int J Oncol. 2018;52(4):1057-70. https://doi.org/10.3892/ijo.2018.4270.
11. Flynn AB, Schiemann WP. Autophagy in breast cancer metastatic dormancy: Tumor suppressing or tumor promoting functions? J Cancer Metastasis Treat. 2019;5. https://doi.org/10.20517/2394-4722.2019.13.
12. Hwang HJ, Ha H, Lee BS, Kim BH, Song HK, Kim YK. *LC3b* is an rna-binding protein to trigger rapid mrna degradation during autophagy. Nat Commun. 2022;13(1):1436.
13. Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, et al. Endogenous hmgb1 regulates autophagy. J Cell Biol. 2010;190(5):881-92. https://doi.org/10.1083/jcb.200911078.
14. Idowu MO, Kmieciak M, Dumur C, Burton RS, Grimes MM, Powers CN, et al. *CD44*(+)/cd24(-/low) cancer stem/progenitor cells are more abundant in triple-negative invasive breast carcinoma phenotype and are associated with poor outcome. Hum Pathol. 2012;43(3):364-73. https://doi.org/10.1016/j.humpath.2011.05.005.
15. Chang SJ, Ou-Yang F, Tu HP, Lin CH, Huang SH, Kostoro J, et al. Decreased expression of autophagy protein lc3 and stemness (cd44+/cd24-/low) indicate poor prognosis in triple-negative breast cancer. Hum Pathol. 2016;48:48-55. https://doi.org/10.1016/j.humpath.2015.09.034.
16. Sun R, Shen S, Zhang YJ, Xu CF, Cao ZT, Wen LP, et al. Nanoparticle-facilitated autophagy inhibition promotes the efficacy of chemotherapeutics against breast cancer stem cells. Biomaterials. 2016;103:44-55. https://doi.org/10.1016/j.biomaterials.2016.06.038.
17. Hortobagyi GN, Edge SB, Giuliano A. New and important changes in the tnm staging system for breast cancer. Am Soc Clin Oncol Educ Book. 2018;38:457-67. https://doi.org/10.1200/EDBK_201313.
18. Roosta Y, Sanaat Z, Nikanfar AR, Dolatkhah R, Fakhrjou A. Predictive value of cd44 for prognosis in patients with breast cancer. Asian Pac J Cancer Prev. 2020;21(9):2561-7. https://doi.org/10.31557/APJCP.2020.21.9.2561.
19. Zhao H, Yang M, Zhao J, Wang J, Zhang Y, Zhang Q. High expression of *LC3B* is associated with progression and poor outcome in triple-negative breast cancer. Med Oncol. 2013;30:1-8.
20. Louhichi T, Ziadi S, Saad H, Dhiab MB, Mestiri S, Trimeche M. Clinicopathological significance of cancer stem cell markers cd44 and aldh1 expression in breast cancer. Breast Cancer. 2018;25(6):698-705. https://doi.org/10.1007/s12282-018-0875-3.
21. Smith AG, Macleod KF. Autophagy, cancer stem cells and drug resistance. J Pathol. 2019;247(5):708-18. https://doi.

org/10.1002/path.5222.
22. Farida A, Yuliantini V. Correlation cd24 and cd44 expression against aggressiveness breast cancer. J Phys Conf Ser. 2019;1246(1):012012.
23. Wei W, Hu H, Tan H, Chow LW, Yip AY, Loo WT. Relationship of cd44+cd24-/low breast cancer stem cells and axillary lymph node metastasis. J Transl Med. 2012;10 Suppl 1(Suppl 1):S6. https://doi.org/10.1186/1479-5876-10-S1-S6.
24. Rustamadji P, Wiyarta E, Bethania KA. Cd44 variant exon 6 isoform expression as a potential predictor of lymph node metastasis in invasive breast carcinoma of no special type. Int J Breast Cancer. 2021;21(8):91-7.
25. Tsang JY, Huang YH, Luo MH, Ni YB, Chan SK, Lui PC, et al. Cancer stem cell markers are associated with adverse biomarker profiles and molecular subtypes of breast cancer. Breast Cancer Res Treat. 2012;136(2):407-17. https://doi.org/10.1007/s10549-012-2271-6.
26. Jang MH, Kang HJ, Jang KS, Paik SS, Kim WS. Clinicopathological analysis of cd44 and cd24 expression in invasive breast cancer. Oncol Lett. 2016;12(4):2728-33. https://doi.org/10.3892/ol.2016.4987.
27. Olsson E, Honeth G, Bendahl PO, Saal LH, Gruvberger-Saal S, Ringner M, et al. Cd44 isoforms are heterogeneously expressed in breast cancer and correlate with tumor subtypes and cancer stem cell markers. BMC Cancer. 2011;11(5):418. https://doi.org/10.1186/1471-2407-11-418.
28. Hassn Mesrati M, Syafruddin SE, Mohtar MA, Syahir A. Cd44: A multifunctional mediator of cancer progression. Biomolecules. 2021;11(12):1850. https://doi.org/10.3390/biom11121850.
29. Jang MH, Kim HJ, Kim EJ, Chung YR, Park SY. Expression of epithelial-mesenchymal transition–related markers in triple-negative breast cancer: Zeb1 as a potential biomarker for poor clinical outcome. Hum Pathol. 2015;46(9):1267-74.
30. de Beca FF, Caetano P, Gerhard R, Alvarenga CA, Gomes M, Paredes J, et al. Cancer stem cells markers cd44, cd24 and aldh1 in breast cancer special histological types. J Clin Pathol. 2013;66(3):187-91. https://doi.org/10.1136/jclinpath-2012-201169.
31. Mustafa MF, Saliluddin SM, Fakurazi S, Tizen Laim NMS, Md Pauzi SH, Nik Yahya NH, et al. Expression of autophagy and mitophagy markers in breast cancer tissues. Front Oncol. 2021;11:612009. https://doi.org/10.3389/fonc.2021.612009.
32. Jain K, Paranandi KS, Sridharan S, Basu A. Autophagy in breast cancer and its implications for therapy. Am J Cancer Res. 2013;3(3):251-65.
33. Su Z, Yang Z, Xu Y, Chen Y, Yu Q. Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer. 2015;14:48. https://doi.org/10.1186/s12943-015-0321-5.
34. Choi J, Jung W, Koo JS. Expression of autophagy-related markers beclin-1, light chain 3a, light chain 3b and p62 according to the molecular subtype of breast cancer. Histopathology. 2013;62(2):275-86. https://doi.org/10.1111/his.12002.
35. Zhou Y, Rucker EB, 3rd, Zhou BP. Autophagy regulation in the development and treatment of breast cancer. Acta Biochim Biophys Sin (Shanghai). 2016;48(1):60-74. https://doi.org/10.1093/abbs/gmv119.
36. Yang MC, Wang HC, Hou YC, Tung HL, Chiu TJ, Shan YS. Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. Mol Cancer. 2015;14:179. https://doi.org/10.1186/s12943-015-0449-3.
37. Wong MM, Chan HY, Aziz NA, Ramasamy TS, Bong JJ, Ch'ng ES, et al. Interplay of autophagy and cancer stem cells in hepatocellular carcinoma. Mol Biol Rep. 2021;48(4):3695-717. https://doi.org/10.1007/s11033-021-06334-9.
38. Han H, Li J, Feng X, Zhou H, Guo S, Zhou W. Autophagy-related genes are induced by histone deacetylase inhibitor suberoylanilide hydroxamic acid via the activation of cathepsin b in human breast cancer cells. Oncotarget. 2017;8(32):53352-65. https://doi.org/10.18632/oncotarget.18410.