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ORIGINAL ARTICLE

ALDH 1A1 and caveolin-1 expression in triple negative breast cancer

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Abstract

Objective Triple negative breast cancer (TNBC) contains a high proportion of breast cancer stem cells (BCSCs) and exhibits resistance to chemotherapy treatments. Therefore, the identification of BCSCs that are novel molecular targets may improve patient survival. Aldehyde dehydrogenase-1 (ALDH 1A1) has been considered a cancer stem cell marker in different tumors. Caveolin-1 (Cav-1), a membrane transporter protein, regulates cancer chemo-resistance and stem cell signaling. Thus, the aim of this study was to evaluate the expression of ALDH 1A1 and Cav-1 in patients with TNBC by immunohistochemistry (IHC) and to correlate their expression with clinical and pathological parameters.

Methods Paraffin blocks of 30 breast cancer patients who underwent modified radical mastectomy between January 2013 and December 2016 in Zagazig University Hospitals (Egypt) were evaluated. Antibodies to ALDH 1A1 and Cav-1 were used.

Results ALDH 1A1 and Cav-1 significantly correlated with tumor size. A significant association between ALDH 1A1/Cav-1 IHC staining and relapse was found. Cav-1 and ALDH 1A1-positive expression correlated with a short 3-year disease-free survival rate and a 3-year overall survival rate ($P < 0.001$).

Conclusion ALDH 1A1 and Cav-1 expression in TNBC was significantly positively correlated with poor clinicopathological parameters and shortened survival. Expression of both markers was significantly positively correlated with each other ($P < 0.001$). ALDH 1A1 and Cav-1 could be potential therapeutic targets in breast cancer.

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The cancer stem cell hypothesis rests on the premise that tumors are composed of tumor cells and a subset of tumor-initiating cells that has the capacity to self-renew and differentiate into multiple cell types, contributing to drug resistance and promoting tumor recurrence or metastasis [1]. Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer with a heterogeneous outcome characterized by the negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2); therefore, TNBC cannot benefit from endocrine therapy and HER-2-targeted therapy [2]. In addition, TNBC exhibits resistance to chemotherapy treatments. Thus, there is a need to find novel drugs that efficiently target this type of breast cancer [3].

Cancer stem cells (CSCs) are a subset of tumor cells that have been thought to contribute to the heterogeneous nature of cancers. Similar to normal stem cells, CSCs have the capacity for indefinite self-renewal and differentiation [4]. Evidence supports the cancer stem cell hypothesis for solid tumors, including breast cancer [5]. In Egypt, breast cancer represents 23.9% of the total malignancies [6].

Locally advanced breast cancer represents a primary tumor with or without extensive regional lymph node metastases. A multimodality treatment approach is usually required to obtain an optimal control of local, regional, and distant disease. The protocol of combined-modality therapy is individualized over a wide range of clinical scenarios, ranging from surgery followed by adjuvant chemotherapy to neoadjuvant chemotherapy

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followed by surgery. In all cases, radiation therapy is tailored to the extent of disease at initial presentation [7].

Cytotoxic chemotherapy continues to be the foundation of current treatment strategies for TNBC because of the lack of known specific therapeutic targets. Third-generation chemotherapy regimens using poly-chemotherapy administered in a dense dose or metronomic manner are the most effective and specific adjuvant treatments for TNBC [8]. Aldehyde dehydrogenase 1A1 (ALDH 1A1) is a key element in the retinoic acid signaling pathway that regulates the self-renewal and differentiation of stem cells [9]. Expression of ALDH 1A1 has been seen in stromal cells and epithelial cells of breast tumors. Tumor environment has a role in the prognostic value of stem cells [10].

Caveolins comprise a family of three proteins: caveolin-1 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3). Cav-1 and Cav-2 have high expression levels in epithelial and endothelial cells, adipocytes, fibroblasts, and pneumocytes. Cav-1 represents an important cancer cell marker in carcinogenesis, tumor progression, and angiogenesis and correlates with resistance to chemotherapy [11]. It was found in a subset of epithelial and mesenchymal cells of normal breast tissue, and the protein has been shown to be associated with the triple-negative immune-phenotype [12]. The goal of this study was to assess the expression of ALDH 1A1 and Cav-1 in patients with TNBC using immunohistochemistry and to correlate their expression with clinical and pathological parameters.

Patients and methods

From January 2013 to December 2016, 30 patients with TNBC aged 29 to 60 years who underwent modified radical mastectomy and admitted to the clinical oncology and nuclear medicine departments of Zagazig University (Egypt) for adjuvant therapy were enrolled in the study. None of the patients underwent treatment before surgery. All patients were administered a chemotherapy regimen (AC-Taxol): doxorubicin (Adriamycin) and cyclophosphamide (Endoxan) repeated every 21 days for four cycles and then paclitaxel (Taxol) weekly for 12 weeks. All patients received locorgional radiotherapy with a total dose of 50 Gy over 5 weeks. Clinical data were collected, including age, menopausal state, history of breast feeding, parity, family history of breast cancer, and tumor size.

Paraffin blocks were obtained from each patient at the pathology department of Zagazig University. Tumor tissues were mounted on charged slides and subjected to immunohistochemical staining via the avidin-biotin peroxidase method using primary monoclonal rabbit anti-ALDH 1A1 antibody (Cat. from Thermo Scientific/Lab Vision Corporation, Fermont, CA, USA, clone: EP1933Y, and 0.09% sodium azide, dilution 1:200) and monoclonal mouse anti-caveolin-1 antibody (Cat. from Thermo scientific/Lab Vision Corporation, Fermont, CA, USA, clone: 18c9, and 0.09% sodium azide, dilution 1:200). For ALDH 1A1, a semi-quantitative evaluation was performed in which the percentage (P) of positive cells (score 0 for 0%, 1 for ≤ 1%, 2 for 1%–10%, 3 for 10%–33%, 4 for 33%–66%, and 5 for 66%–100% positive cells) and the intensity (I) of staining (score 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining) were included, and a Quick score was generated (Q = P + I; score range: 0–8) [13]. For Cav-1, cases were classified as positive if membranous staining of ≥ 5% of tumor cells was identified as positive [14]. Stromal immunoreactivity in the neoplastic and adjacent non-neoplastic breast tissue was observed, but not incorporated in the scoring of either marker's expression.

Statistical analysis

Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Percentages of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when appropriate. Trend of change in distribution of relative frequencies between ordinal data was compared using the Chi-square test for trend. Disease free survival (DFS) was calculated from the time of surgery to relapse or to the most recent follow-up at which the patient was free from relapse. Overall survival (OS) was calculated as the time from diagnosis to death or to the most recent follow-up contact (censored). Stratification of DFS and OS was done according to immunohistochemical markers. These time-to-event distributions were estimated using the method of the Kaplan-Meier plot and compared using a two-sided exact log-rank test. All tests were two sided. A P-value < 0.05 was considered significant. All statistics were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc for Windows (MedCalc Software bvba 13, Ostend, Belgium).

Results

Patients' characteristics

The age of patients (n=35) at the time of initial diagnosis ranged from 26 to 64 years. The mean and median ages were (47.9 ± 10.1) years and 42 years, respectively. The median follow-up time was 36 months (range: 12–36 moths), and during the follow-up period, 13.3% of the patients were disease-free without relapse or metastasis. Recurrence and/or metastasis occurred in 86.7% of the patients, and 43.3% of these patients (13/30) died during follow-up. The clinicopathological characteristics of the

Table 1 Clinicopathological features, immunohistochemical markers, and outcome of 30 patients with triple negative breast cancer

Characteristics	All (n = 30)	
	No.	%
T		
T1	10	33.3%
T2	6	20.0%
T3	10	33.3%
T4	4	13.3%
N		
N0	10	33.3%
N1	10	33.3%
N2	10	33.3%
AJCC stage grouping		
Stage IIB	12	40.0%
Stage IIIA	14	46.7%
Stage IIIB	4	13.3%
Stage II	12	40.0%
Stage III	18	60.0%
ALDH 1A1		
Negative	10	33.3%
Positive	20	66.7%
Caveolin 1		
Negative	15	50.0%
Positive	15	50.0%
ALDH 1A1/Caveolin 1		
Negative/Negative	10	33.3%
Positive/Negative	5	16.7%
Positive/Positive	15	50.0%
Follow-up		
Mean ± SD	33.20 ± 6.27	
Median (range, month)	36 (12 – 36)	
Relapse		
Absent	4	13.3%
Present	26	86.7%
Mortality		
Alive	17	56.7%
Died	13	43.3%

Note: Categorical variables are expressed as number (percentage). Continuous variables are expressed as mean ± SD & median (range)

30 patients with TNBC were summarized in Table 1.

Association of ALDH 1A1 and Cav-1 expression with clinicopathological parameters (Tables 2 and 3)

Positive ALDH 1A1 expression was observed in 66.6% of the patients. Positive Cav-1 IHC staining was observed in 50% of the patients. ALDH 1A1 was stained in the cytoplasm of cancer cells (Fig. 1). Cav-1 was stained in the membranes of cancer cells (Fig. 2). Both markers were significantly correlated with tumor size T (P = 0.01). In addition, a significant association was observed between T and ALDH 1A1 IHC staining, with 75% of the patients with T4 disease having a positive staining versus 50% of the patients with T1 disease.

A significant association between T and ALDH 1A1/Cav-1 IHC staining was observed, in which 50% of the patients with T4 disease had positive/positive staining versus 30% of the patients with T1 disease. No significant association between IHC staining of either marker, N, and the AJCC stage grouping was observed. There was a significant association between ALDH 1A1 and Cav-1 IHC staining, in which 75% of the patients with positive staining for ALDH 1A1 showed a positive staining for Cav-1 versus 100% of the patients with negative staining for ALDH 1A1 showed a negative staining for Cav-1.

Association between ALDH 1A1 and Cav-1 expression and tumor relapse (Table 4)

A significant association between ALDH 1A1/Cav-1 IHC staining and relapse was observed, in which 100% of the patients with positive/positive staining relapsed versus 70% of the patients with negative/negative staining who showed no significant association among the T, N, AJCC stage grouping, ALDH 1A1/Cav-1 staining, and relapse.

Association between ALDH 1A1 and Cav-1 expression and survival (Tables 5 and 6; Fig. 3)

A significant difference between patients with negative ALDH 1A1 expression and those with positive ALDH 1A1 expression with respect to DFS, where the mean DFS for patients with negative expression was longer than the mean DFS for those with positive expression (33.4 versus 29.3 months, P = 0.041) and the 3-year DFS was 30% versus 0%, respectively. A significant association between ALDH 1A1/Cav-1 IHC staining and mortality was found, in which 66.7% of the patients with positive/positive staining died versus 10% of patients with negative/negative staining. A significant difference among positive/positive staining patients, negative/negative staining patients, and positive/negative staining patients with respect to OS was observed, where the mean OS for negative/negative patients was longer than the mean OS for positive/positive patients (36 versus 32.7 months, P = 0.032) and the 3-year OS was 88.9% versus 33.3%, respectively.

A significant association between ALDH 1A1 IHC staining and mortality was observed, in which 60% of the patients with positive staining died versus 10% of the patients with negative staining, and a significant association between Cav-1 IHC staining and mortality was observed, in which 66.7% of the patients with positive staining died versus 20% of patients with negative staining. A significant difference between patients with negative ALDH 1A1 expression and those with positive ALDH 1A1 expression with respect to OS was observed, where the mean OS for negative patients was longer than the mean OS for positive patients (36 versus 33.2

Table 2 Relation between clinicopathological features and immunohistochemical markers of 30 patients with triple negative breast cancer

Characteristics	All (n = 30)	ALDH 1A1			Caveolin 1		
		Negative (n = 10)	Positive (n = 20)	P-value	Negative (n = 15)	Positive (n = 15)	P-value
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	
Tumor size (mm)							
< 20	10 (33.3%)	0 (0%)	10 (100%)	0.015*	2 (20.0%)	8 (80.0%)	0.023*
> 20	20 (66.7%)	10 (50.0%)	10 (50.0%)		13 (65.0%)	7 (35.0%)	
T							
T1	10 (33.3%)	5 (50.0%)	5 (50.0%)	0.042**	7 (70.0%)	3 (30.0%)	0.043**
T2	6 (20.0%)	4 (66.7%)	2 (33.3%)		5 (83.3%)	1 (16.7%)	
T3	10 (33.3%)	0 (0%)	10 (100%)		1 (10.0%)	9 (90.0%)	
T4	4 (13.3%)	1 (25.0%)	3 (75.0%)		2 (50.0%)	2 (50.0%)	
N							
N0	10 (33.3%)	1 (10.0%)	9 (90.0%)	0.062**	3 (30.0%)	7 (70.0%)	0.079**
N1	10 (33.3%)	4 (40.0%)	6 (60.0%)		5 (50.0%)	5 (50.0%)	
N2	10 (33.3%)	5 (50.0%)	5 (50.0%)		7 (70.0%)	3 (30.0%)	
AJCC stage grouping							
Stage IIB	12 (40.0%)	4 (33.3%)	8 (66.7%)	0.852**	6 (50.0%)	6 (50.0%)	1.000**
Stage IIIA	14 (46.7%)	5 (35.7%)	9 (64.3%)		7 (50.0%)	7 (50.0%)	
Stage IIIB	4 (13.3%)	1 (25.0%)	3 (75.0%)		2 (50.0%)	2 (50.0%)	
Stage II	12 (40.0%)	4 (33.3%)	8 (66.7%)	1.000*	6 (50.0%)	6 (50.0%)	1.000*
Stage III	18 (60.0%)	6 (33.3%)	12 (66.7%)		9 (50.0%)	9 (50.0%)	
ALDH 1A1							
Negative	10 (33.3%)				10 (100%)	0 (0%)	< 0.001*
Positive	20 (66.7%)				5 (25.0%)	15 (75.0%)	
Caveolin							
Negative	15 (50.0%)	10 (66.7%)	5 (33.3%)	< 0.001*			
Positive	15 (50.0%)	0 (0%)	15 (100%)				

Note: Categorical variables are expressed as number (percentage). Mann Whitney U test; * Chi-square test; ** Chi-square test for trend; P < 0.05 is significant

Table 3 Relation between clinicopathological features and immunohistochemical markers of 30 patients with triple negative breast cancer

Characteristics	All (n = 30)	ALDH 1A1/Caveolin 1			P-value
		Negative/Negative (n = 10)	Positive/Negative (n = 5)	Positive/Positive (n = 15)	
	No. (%)	No. (%)	No. (%)	No. (%)	
T					
T1	10 (33.3%)	5 (50.0%)	2 (20.0%)	3 (30.0%)	0.028**
T2	6 (20.0%)	4 (66.7%)	1 (16.7%)	1 (16.7%)	
T3	10 (33.3%)	0 (0%)	1 (10.0%)	9 (90.0%)	
T4	4 (13.3%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	
N					
N0	10 (33.3%)	1 (10.0%)	2 (20.0%)	7 (70.0%)	0.050**
N1	10 (33.3%)	4 (40.0%)	1 (10.0%)	5 (50.0%)	
N2	10 (33.3%)	5 (50.0%)	2 (20.0%)	3 (30.0%)	
AJCC stage grouping					
Stage IIB	12 (40.0%)	4 (33.3%)	2 (16.7%)	6 (50.0%)	0.988**
Stage IIIA	14 (46.7%)	5 (35.7%)	2 (14.3%)	7 (50.0%)	
Stage IIIB	4 (13.3%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	
Stage II	12 (40.0%)	4 (33.3%)	2 (16.7%)	6 (50.0%)	1.000*
Stage III	18 (60.0%)	6 (33.3%)	3 (16.7%)	9 (50.0%)	

Note: Categorical variables are expressed as number (percentage). * Chi-square test; ** Chi-square test for trend; P < 0.05 is significant

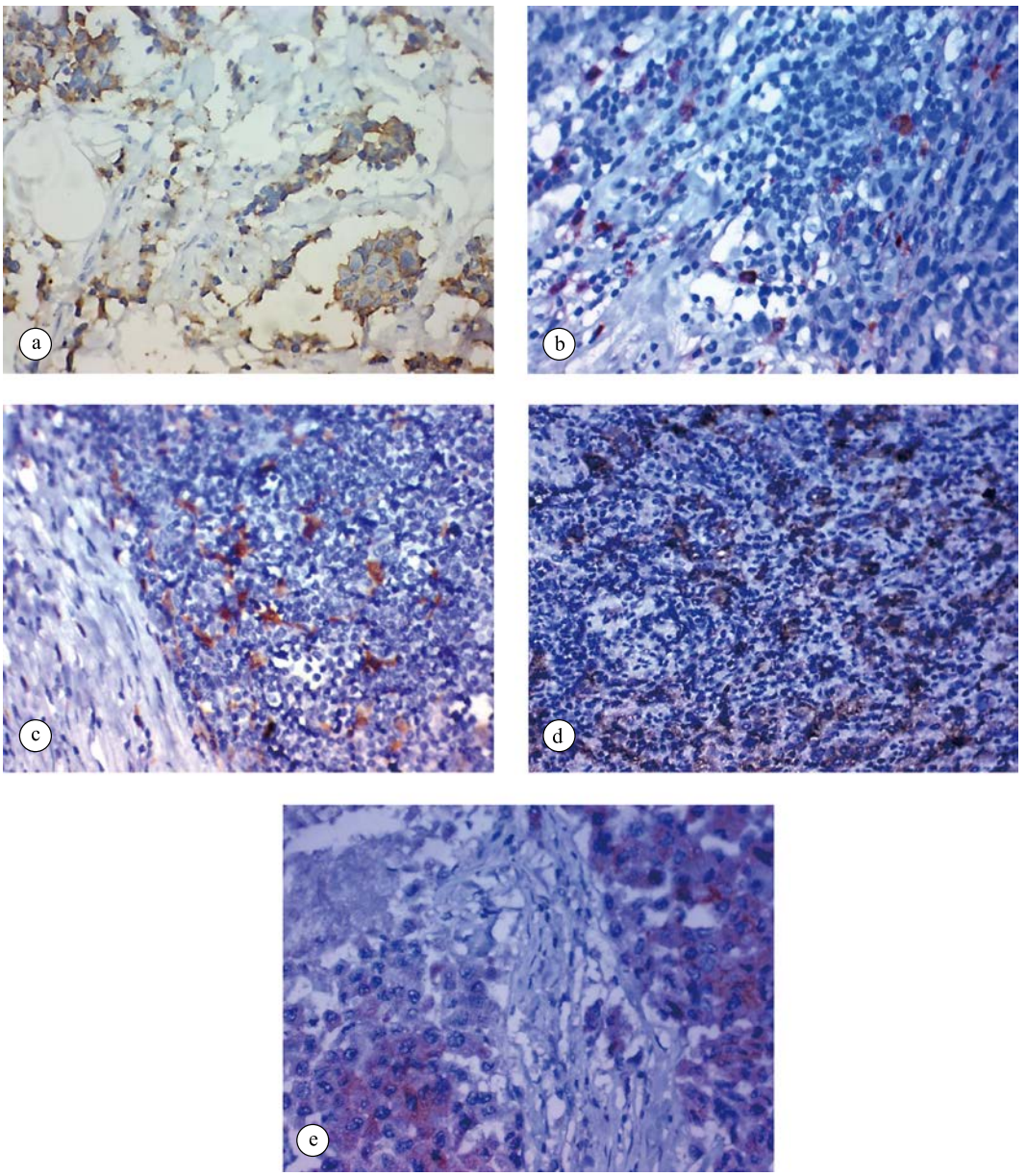

Fig. 1 Immunohistochemical staining of ALDH 1A1, infiltrating duct carcinoma cytoplasmic expression. (a) abundant tumor cells with moderate staining intensity (score = 2 + 4; P + I). (b) few tumor cells with moderate staining intensity (score = 1 + 2; P + I). (c) abundant tumor cells with moderate staining intensity (score = 2 + 4; P + I; IHC x 200). (d) abundant tumor cells with moderate staining intensity (score = 2 + 4; P + I; IHC x 100). (e) abundant tumor cells with moderate staining intensity (score = 2 + 4; P + I; IHC x 400)

months, $P = 0.008$) and 3-year OS was 88.9% versus 34%, respectively. A significant difference between negative Cav-1 patients and positive Cav-1 patients with respect to OS, where the mean OS for negative patients was longer than the mean OS for positive patients (35.6 versus 32.7 months, $P = 0.030$) and the 3-year OS was 75.9% versus 33.3%, respectively.

Discussion

CSCs have been shown to be involved in the initiation, progression, and recurrence of cancer [15]. The American Society of Clinical Oncology and the American College of Pathology have defined TNBC as breast cancer with less than 1% of tumor cells expressing the ERs and PRs

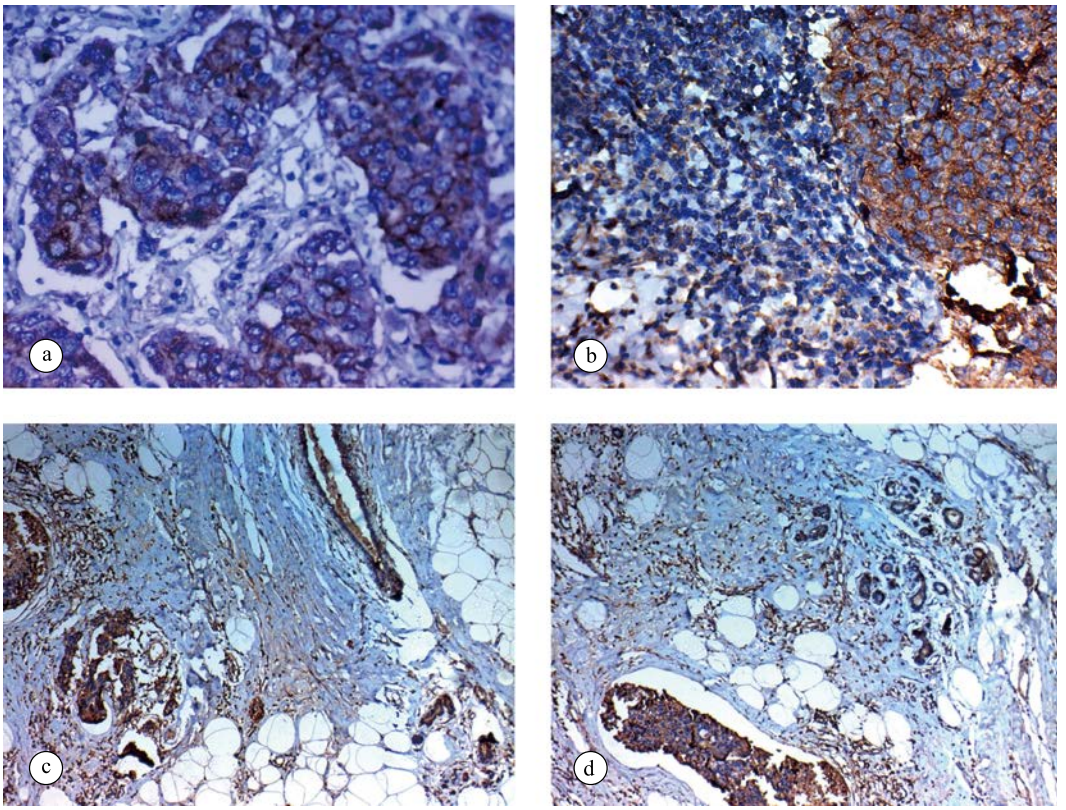

Fig. 2 (a and b) Immunohistochemical staining of infiltrating duct carcinoma membranous expression for caveolin-1 (IHC x 400). (c and d) Immunohistochemical staining of Infiltrating duct carcinoma and also in fat, blood vessels and normal breast tissue expression for caveolin-1 (IHC x 200)

via IHC [16]. TNBC is an aggressive breast cancer subtype with limited treatment options. Identification of novel molecular targets is critical for the development of successful therapies for this very aggressive subtype of breast cancer [3, 17]. TNBC demonstrates a heterogeneous group of cancer, which is enriched in cells with stem cell-like properties [18]. Therefore, molecular targets for CSCs in breast cancer treatment are needed for effective treatments and therapies.

ALDH 1A1+ may be used as biomarker to identify breast cancer stem cell groups and is correlated with the malignant transformation of breast tissue and progression into a more aggressive triple-negative phenotype [19]. Ginestier *et al* have shown that ALDH 1A1 activity leads to increased metastasis through retinoic acid signaling and induces the differentiation of breast CSCs [20]. In addition, ALDH 1A1 acts as a detoxifying enzyme and mediator of progenitor cell expansion and differentiation.

Cav-1, a membrane transporter protein, is involved in the regulation of cancer chemotherapy resistance and stem cell signaling and is highly elevated in patients with TNBC [21]. In the present study, positive ALDH 1A1 expression was observed in 66.6% of the patients and ALDH 1A1 was expressed in both epithelial tumor cells and stromal cells, which can be explained by its role in stem cells and cellular differentiation. This is supported by the work of Ohi *et al*, who found ALDH 1A1 expression in malignant cells of 59% of the cases [22], which is slightly lower than the percentage reported by Madjd *et al*, who found expression of ALDH 1A1 in 74% of cases [23]. These differences may be attributed to different scoring methods used by the authors.

Regarding tumor size, there was a significant positive correlation between ALDH 1A1 expression and tumor size (P= 0.01), which is similar to the findings of Yoshioka *et al*, who reported that ALDH 1A1 expression was correlated with larger tumor size [24]. However, these findings are in disagreement with the results of Neumeister *et al* [25] and Madjd *et al* [23], who were unable to verify significant correlations between expression of ALDH 1A1 and tumor size. These outcome differences may be a result of varying sample sizes. In regards to the histological grade, this study revealed a non-significant correlation between ALDH 1A1 expression and histological grade. This is similar to other studies conducted by Murrjag *et al* [26], Hosni *et al* [27], Resetkova *et al* [10], and Zhou *et al* [28]. However, Ginestier *et al* [20] and Ricardo *et al* [29] reported that ALDH 1A1 was related to a high histological grade.

Table 4 Relation between clinicopathological features/immunohistochemical staining and relapse/mortality of 30 patients with triple negative breast cancer

Characteristics	All (*n* = 30)	Relapse		*P*-value	Mortality		*P*-value
		Absent (*n* = 4)	Present (*n* = 26)		Alive (*n* = 17)	Died (*n* = 13)	
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	
T							
T1	10 (33.3%)	1 (10.0%)	9 (90.0%)	0.643**	7 (70.0%)	3 (30.0%)	0.026**
T2	6 (20.0%)	1 (16.7%)	5 (83.3%)		6 (100%)	0 (0%)	
T3	10 (33.3%)	1 (10.0%)	9 (90.0%)		3 (30.0%)	7 (70.0%)	
T4	4 (13.3%)	1 (25.0%)	3 (75.0%)		1 (25.0%)	3 (75.0%)	
N							
N0	10 (33.3%)	2 (20.0%)	8 (80.0%)	0.518**	4 (40.0%)	6 (60.0%)	0.183**
N1	10 (33.3%)	1 (10.0%)	9 (90.0%)		6 (60.0%)	4 (40.0%)	
N2	10 (33.3%)	1 (10.0%)	9 (90.0%)		7 (70.0%)	3 (30.0%)	
AJCC stage grouping							
Stage IIB	12 (40.0%)	2 (16.7%)	10 (83.3%)	0.959**	9 (75.0%)	3 (25.0%)	0.065**
Stage IIIA	14 (46.7%)	1 (7.1%)	13 (92.9%)		7 (50.0%)	7 (50.0%)	
Stage IIIB	4 (13.3%)	1 (25.0%)	3 (75.0%)		1 (25.0%)	3 (75.0%)	
Stage II	12 (40.0%)	2 (16.7%)	10 (83.3%)	1.000*	9 (75.0%)	3 (25.0%)	0.098*
Stage III	18 (60.0%)	2 (11.1%)	16 (88.9%)		8 (44.4%)	10 (55.6%)	
ALDH 1A1							
Negative	10 (33.3%)	3 (30.0%)	7 (70.0%)	0.095*	9 (90.0%)	1 (10.0%)	0.017*
Positive	20 (66.7%)	1 (5.0%)	19 (90.0%)		8 (40.0%)	12 (60.0%)	
Caveolin							
Negative	15 (50.0%)	4 (26.7%)	11 (73.3%)	0.100*	12 (80.0%)	3 (20.0%)	0.010*
Positive	15 (50.0%)	0 (0%)	15 (100%)		5 (33.3%)	10 (66.7%)	
ALDH 1A1/Caveolin1							
Negative/Negative	10 (33.3%)	3 (30.0%)	7 (70.0%)	0.031*	9 (90.0%)	1 (10.0%)	0.006**
Positive/Negative	5 (16.7%)	1 (20.0%)	4 (80.0%)		3 (60.0%)	2 (40.0%)	
Positive/Negative	15 (50.0%)	0 (0%)	15 (100%)		5 (33.3%)	10 (66.7%)	
Relapse							
Absent	4 (13.3%)				4 (100%)	0 (0%)	0.113*
Present	26 (86.7%)				13 (50.0%)	13 (50.0%)	

Note: Categorical variables are expressed as number (percentage). * Chi-square test; ** Chi-square test for trend; $P < 0.05$ is significant

In the present study, the analysis of DFS showed a significant difference among ALDH 1A1-positive and ALDH 1A1-negative tumors. This result was similar to that of Shima *et al* [30], who reported that DFS and OS were significantly lower for ALDH 1A1-positive patients than for ALDH 1A1-negative patients. In contrast, Kim *et al* [31] and Murrja *et al* [26] showed no difference among ALDH 1A1-positive and ALDH 1A1-negative tumors (P= 0.61), in regards to DFS. Such differences may be related to the nature of the study groups and genetic differences. Similar results were obtained by Kim *et al* [31] who showed ALDH 1A1 expression in breast cancer could be correlated with poor prognosis, and may contribute to a more aggressive cancer phenotype.

Li *et al* [32] showed that ALDH 1A1 expression is higher in TNBC than in non-TNBC and associated with a poorer prognosis of TNBC patients. Ohi *et al* [33] found that ALDH 1A1 expression was correlated with high histological grade alone ($P < 0.006$) and a shorter relapse-free survival. Previous researchers who studied ALDH 1A1 expression in most solid human cancers, such as bladder, lung, prostate, pancreatic, and gastric cancer, proved that ALDH 1A1 is correlated with advanced tumor grade, stage, higher recurrence, and shorter survival rates [34–36]. Yang *et al* indicated that ALDH 1A1-positive breast cancer cells were associated with the TNBC subtype [37].

Cav-1 has only been evaluated in tumor cells and its expression in the current study was similar to that reported by Savelina *et al* [14] and Savage *et al* [38]. In the current study, Cav-1 expression was reported in 50% of the TNBC cases. Cav-1 was expressed in tumor cells, stromal cells, endothelial cells, myoepithelial cells, and fibroblasts. This is in accordance with previous studies conducted by Perou *et al* [39], Charafe-Jauffret *et al* [40], and Savage *et al* [38, 41]. Engelman *et al* reported that Cav-1 maintained the basal/myoepithelial phenotype and was expressed in basal/myoepithelial cells of normal breast tissue [42].

The mechanisms underlying the expression of Cav-1 in breast cancer, specifically the basal-like phenotype, have

Table 5 Relation between immunohistochemical markers and outcome of 30 patients with triple negative breast cancer

Characteristics	All (*n* = 30)	ALDH 1A1		*P*-value	Caveolin		*P*-value
		Negative (*n* = 10)	Positive (*n* = 20)		Negative (*n* = 15)	Positive (*n* = 15)	
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	
Relapse							
Absent	4 (13.3%)	3 (30.0%)	1 (5.0%)	0.095*	4 (26.7%)	0 (0%)	0.100*
Present	26 (86.7%)	7 (70.0%)	19 (95.0%)		11 (73.3%)	15 (100%)	
Disease Free Survial (DFS)							
Mean (month)	30.7	33.4	29.3	0.041**	29.3	29.7	0.359**
95% CI	27.61–33.83	29.91–36.89	25.03–33.62		28.42–35.01	24.4–35.05	
HR (95% CI)		1.847 (0.776–4.396)			1.288 (0.591–2.805)		
1-year DFS	93.2%	100%	89.7%		92.9%	80.0%	
2-year DFS	75.9%	80.0%	73.9%		78.6%	73.3%	
3-year DFS	10.4%	30.0%	0%		21.4%	0%	
Mortality							
Alive	17 (56.7%)	9 (90.0%)	8 (40.0%)	0.017*	12 (80.0%)	5 (33.3%)	0.010*
Died	13 (43.3%)	1 (10.0%)	12 (60.0%)		3 (20.0%)	10 (66.7%)	
Overall Survival (OS)							
Mean (month)	34.1	36	33.2	0.008**	35.6	32.7	0.030**
95% CI	32.08–36.16		30.17–36.14		34.58–36.56	28.78–36.56	
HR (95% CI)		7.405 (0.962–56.970)			3.217(0.885–11.697)		
1-year OS	96.7%	100%	95.0%		100%	93.3%	
2-year OS	93.3%	100%	90.0%		100%	86.7%	
3-year OS	52.6%	88.9%	34.0%		75.9%	33.3%	

Note: Categorical variables are expressed as number (percentage); continuous variables were expressed as mean (95% CI). * Chi-square test; ** Log rank test; 95% CI: 95% Confidence Interval; $P < 0.05$ is significant

Table 6 Relation between immunohistochemical markers and outcome of 30 patients with triple negative breast cancer

Characteristics	All (*n* = 30)	ALDH 1A1/Caveolin 1			*P*-value
		Negative/Negative (*n* = 10)	Positive/Negative (*n* = 5)	Positive/Positive (*n* = 15)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Relapse					
Absent	4 (13.3%)	3 (30.0%)	1 (20.0%)	0 (0%)	0.031*
Present	26 (86.7%)	7 (70.0%)	4 (80.0%)	15 (100%)	
Disease free survial (DFS)					
Mean (month)	30.7	33.4	27.5	29.7	0.061**
95% CI	27.61–33.83	29.91–36.89	21.02–33.98	24.42–35.05	
1-year DFS	93.2%	100%	100%	86.7%	
2-year DFS	75.9%	80.0%	75.0%	73.3%	
3-year DFS	10.4%	30.0%	0%	0%	
Mortality					
Alive	17 (56.7%)	9 (90.0%)	3 (60.0%)	5 (33.3%)	0.006*
Died	13 (43.3%)	1 (10.0%)	2 (40.0%)	10 (66.7%)	
Disease free survial (OS)					
Mean (month)	34.1	36	34.5	32.7	0.032**
95% CI	32.08–36.16		30.90–38.10	28.78–36.56	
1-year OS	96.7%	100%	100%	93.3%	
2-year OS	93.3%	100%	100%	86.7%	
3-year OS	52.6%	88.9%	37.5%	33.3%	

Note: Categorical variables are expressed as number (percentage); continuous variables were expressed as mean (95% CI). * Chi-square test; ** Log rank test; 95% CI: 95% Confidence Interval; $P < 0.05$ is significant

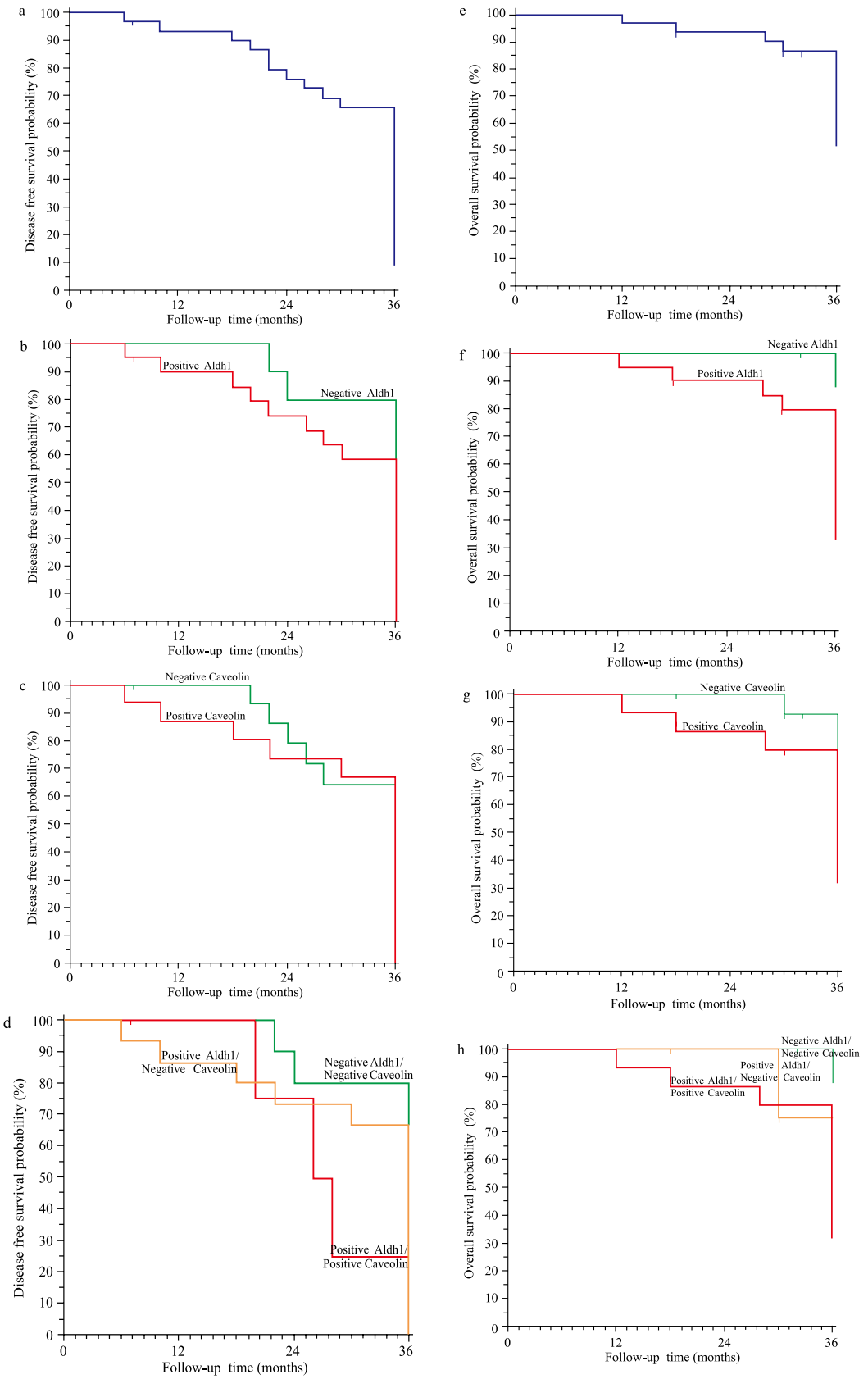

Fig. 3 Kaplan-Meier survival plots. Left panel: disease free survival; Right panel: overall survival. (a & e) All studied triple negative breast cancer patients; (b & f) Stratified by ALDH 1A1 IHC staining; (c & g) Stratified by Caveolin IHC staining; (d & h) Stratified by Aldh-1/Caveolin IHC staining

yet to be elucidated, but most likely could be a result of gene amplification [38, 41]. Hypomethylation also occurs, which has been reported by Kagara *et al,* who found a significant positive correlation between Cav-1 expression and a high histological grade and between lymph node metastasis and large tumor size (P = 0.02, 0.03, and < 0.001, respectively) [43]. These results are similar to those of Salatino *et al*, who explained that Cav-1 has been demonstrated to mediate medroxyprogesterone acetate-(MPA)-induced breast cancer cell growth [44].

Similar studies conducted by Zuccari *et al* [45] and Savage *et al* [38] identified a positive correlation between the expression of Cav-1 and high histological grade. Similarly, Sagara *et al* found that reduced Cav-1 mRNA levels using real-time polymerase chain reaction significantly correlates with increasing tumor size (P = 0.041) [46].

Cav-1 expression was significantly associated with highly aggressive tumors, such as inflammatory breast carcinoma [47], basal-like carcinoma [38, 41], and TNBC [38, 41, 48-49]. In this study, patients with Cav-1-positive cancers had a shorter DFS. This can be explained by the fact that Cav-1 expression in human cancer cells serves as a tumor promoter and upregulates Cav-1 in late stage disease, which may promote resistance against chemo-therapeutic agents [50]. Elsheikh *et al* reported similar results, demonstrating that a positive expression of Cav-1 was associated with a shorter DFS. Koo *et al* reported similar results [51] and El-Gendi and Mostafa showed that approved tumor epithelial cell Cav-1 positive staining was not associated with survival and patient outcome [52]. However, the results were different from those of Simpkins *et al* [53], Witkiewicz *et al* [54], and Howell *et al* [55], who found a positive correlation between positivity of Cav-1 in the tumor stroma and increased OS. In regards to positive marker expression and tumor relapse, a significant association was found between ALDH 1A1/ Cav-1 IHC staining and relapse, which is in agreement with previous studies by Zhong *et al* [56] and Elsheikh *et al* [12].

In the current study, there was a significant positive correlation between positive expressions of both ALDH 1A1 and Cav-1 in TNBC (P< 0.001). This can be explained by the fact that Cav-1 acts as a stem cell signal and Cav-1 silencing could sensitize breast CSCs by limiting their self-renewal and induction of differentiation, as reported by Wang *et al* [21]. Shajahan *et al* demonstrated that Cav-1 expression is correlated with TNBC; therefore, Cav-1 expression can be used to select patients with TNBC who may benefit from dasatinib treatment [57]. In addition, ALDH 1A1-positive cancer cells are highly tumorigenic, and suppression of ALDH 1A1 leads to lower tumorigenicity [56].

Conclusion

Expression of both ALDH 1A1 and Cav-1 in TNBC was significantly positively correlated with parameters indicating poor clinical pathology and poor prognosis and expression of both of them were significantly positively correlated with each other (P < 0.001). Further, ALDH 1A1 and Cav-1 expression was significantly associated with shortened 3-year DFS and 3-year OS (P < 0.001). ALDH 1A1 and Cav-1 could be potential therapeutic targets in breast cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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