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RESEARCH

# Stromal Caveolin-1 Expression in Breast Carcinoma. Correlation with Early Tumor Recurrence and Clinical Outcome

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**Abstract** Caveolin- (cav-1) has been linked to tumor progression and clinical outcome in breast cancer, but its role as a prognostic marker is still unclear. We evaluated stromal and tumor caveolin-1 expression in 91 breast carcinomas, and assessed the association between their expression and clinicopathologic variables as well as patient outcome and early tumor recurrence. Absence of stromal caveolin-1 expression was detected in 18.7% of cases, while 25.3% of cases revealed tumor epithelial caveolin-1 expression. Combined stromal and tumor caveolin-1 immunopositivity was seen in 24.2% of cases. Absence of stromal cav-1 associated with larger tumor size, higher grade, higher nodal stage, higher number of positive nodes, higher TNM stage, positive HER2 status, higher recurrence rate, and shorter mean progression free survival (PFS). Stromal cav-1 status was a significant predictor of PFS in ER+, PR +, and HER2 + tumors. In tamoxifen-treated patients, absence of stromal Cav-1 was a significant predictor of poor clinical outcome, suggestive of tamoxifen resistance. Conversely, tumor epithelial and combined caveolin-1 expression, didnot associate with patient outcome. In multivariate analysis, only TNM stage independently associated with survival. Loss of stromal caveolin-1 is a novel breast cancer biomarker that can predict early tumor recurrence, short PFS, and tamoxifen- resistance. Thus, its use as a predictive biomarker, especially in lower grade, lower stage, ER+, PR+, HER2+, and tamoxifen treated patients may allow for early interventions with more aggressive therapies. Thus, stromal marker expression and epithelial-stromal cross talk may be critical for tumor progression and metastasis.

**Keywords** Breast carcinoma · Clinical outcome · Predictive value · Stromal caveolin-1 · Tumor recurrence

**Abbreviations**

| | |
|---|---|
| CAFs | Cancer associated fibroblasts |
| Cav-1 | Caveolin-1 |
| DCIS | Ductal carcinoma in situ |
| FFPE | Formalin fixed paraffin embedded |
| MECs | Myoepithelial cells |
| PASW | Predictive analytics software |
| PFS | Progression free survival |

## Introduction

Invasive breast cancer is still the most common female malignancy worldwide and more than one million women are diagnosed with breast cancer each year [1, 2]. Carcinoma cells grow in a complex tumor microenvironment composed of non-epithelial cells (including fibroblasts, pericytes, endothelial, and inflammatory cells), extracellular matrix, and secreted diffusible growth fac-

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tors/cytokines [3–6]. Under normal physiological conditions the stroma serves as an important barrier to malignant transformation, however, its role changes during neoplastic transformation [7].

In recent years, it became apparent that all stromal components in the tumor microenvironment have a profound influence on tumor growth, invasiveness and metastasis [5, 7, 8]. The molecular cross talk between tumor cells and these stromal elements plays an important role in defining the phenotype of a tumor [5]. Tumor cells can trigger the deposition of a reactive stroma or desmoplasia containing activated fibroblasts, connective tissue, immune and inflammatory cells [6]. Conversely, fibroblasts isolated from the tumor stroma can promote tumor growth [4–6, 9]. This population of tissue fibroblasts -that are termed "cancer associated fibroblasts" (CAFs)- is characterized by a hyper-proliferative phenotype, and in addition to inducing cancer cell proliferation, CAFs also show an ability to prevent cancer cell apoptosis, and stimulate tumor angiogenesis [10].

To date, the mechanisms that govern the conversion of benign mammary stromal fibroblasts to tumor-associated fibroblasts are poorly understood [3]. Down-regulation of caveolin-1 (Cav-1) [11] - one of the caveolins [12] - is one of the mechanisms implicated in the oncogenic transformation of fibroblasts [11]. Caveolins are the principal protein component of caveolae (which are flask-shaped invaginations of the plasma membrane with an average diameter of 50–100 nm [13] that are located at the cell surface in most cell types [11].

Cav-1 normally functions as a transformation suppressor that prevents cell cycle progression [3]. It plays a major role in tumorigenesis through its various functions such as lipid transport, membrane trafficking, gene regulation, and signal transduction [12]. Many cell types in the mammary stroma express caveolin-1. However the role of this protein in the molecular cross talk between tumor and stromal cells remains unknown [8].

This study aimed at evaluating the stromal and epithelial tumor cell expression of caveolin-1 in a cohort of breast carcinoma patients, and to assess any association between stromal caveolin-1 expression and clinicopathologic variables as well as patient outcome and early tumor recurrence.

## Material and Methods

Formalin fixed paraffin-embedded (FFPE) tissue blocks derived from 91 consecutive primary breast carcinomas excised at the time of surgery with available complete pathology information and sufficient tissue for immunostaining, were obtained from the files of Surgical Pathology Laboratory, University of Alexandria, Faculty of Medicine. Apart from 17 cases (that were locally advanced at the time of initial presentation), surgery was the first line of treatment in all cases, and samples for analysis were obtained before chemotherapy.

The median age of the study population was 49 years; mean 50.1 ±11.6 years (range, from 27 to 76 years). Follow-up information for patients treated at the Clinical Oncology Department, Main University Hospital, Alexandria Faculty of Medicine, were collected from the computer files and archeives of the Clinical Oncology Department in the period from January 2007 to January 2011. The mean follow-up time for all survivors was 21.94± 10.62 months.

Clinical and pathological variables were determined following well-established criteria. The histological type of primary breast tumor was classified based on Page et al. [14] and the College of American Pathologists recommendations [15]. All invasive carcinomas were graded according to the method described by Ellis and Elston [16]. Clinical and treatment information were gathered by chart review.

### Immunohistochemical Staining

Sequential sections from each case were stained for 4 antibodies (Caveolin-1, ER, PR, and HER2) to allow comparing the staining characteristics of the same group of tumor cells. Immunostaining was done on 5-μm thick sections of FFPE tissue mounted on polylysine-coated microslides, dewaxed and rehydrated. Sections were incubated for 15 min in 3% hydrogen peroxide to quench endogenous tissue peroxidase. Heat induced antigen retrieval was done for all antibodies (30 min for caveolin-1, and 20 min for ER, PR, and HER2) in a microwave oven in 10 mM citrate buffer, pH 6.0). Then, tissue sections were incubated with the primary antibodies. For Caveolin-1, ER, and PR rabbit monoclonal antibodies were used; caveolin-1 (Clone E249,(ab32577), England) at a dilution 1:100, ER (Clone SP1, Thermoscientific, NeoMarkers, Fremont, USA) at a dilution 1:100, and PR (Clone SP2, Thermoscientific, NeoMarkers, Fremont, USA) at a dilution 1:200. HER2 immunostaining was done using a mouse monoclonal antibody (Clone e2-4001 + 3B5, Thermoscientific, NeoMarkers, Fremont, USA) at a dilution 1:200. The antigen-antibody reaction was visualized by Thermo scientific UltraVision LP Detection System. Immunohistochemical reactions were developed with diaminobenzidine and sections were counterstained with Harrris hematoxylin. All immunostains were manually processed, with appropriate external positive and negative controls included for each immunohistochemical run. Furthermore, all sections had internal positive control for cav-1 (vascular endothelial cells and adipocytes), and hormone receptors (breast tissue adjacent to the tumor).

### Quantitation of Immunostaining

Evaluation of immunostained slides was performed in a blinded manner without knowledge of the assigned clinical data. Caveolin-1 staining in both tumor and stromal cells was scored semiquantitatively as: (0) no staining, (1) either diffuse weak or focal strong staining in less than 30% of cells, or (2) defined as strong staining of 30% or more of cells [3], (as regards staining intensity; weak denotes a staining intensity weaker than endothelial cells, and strong denotes a staining intensity similar to endothelial cells). Only membranous with or without cytoplasmic staining was considered specific, and non-neoplastic endothelial cells were used as internal positive controls for immunohistochemical caveolin-1 expression analysis [17]. Accordingly, in our study entrapped vessels served as internal positive control, revealing a positive staining for anti-caveolin-1 antibody.

ER and PR immunostaining were scored according to Allred score [18] which is a semi-quantitative system that takes into consideration the proportion of positive cells, and the intensity of staining. The proportion of positive cells was scored on a scale of 0–5 (0=no nuclear staining, 1=<1% nuclear staining, 2=1–10% nuclear staining, 3=11–33% nuclear staining, 4=34–66% nuclear staining and 5=67–100% nuclear staining) and the staining intensity was scored on a scale of 0–3 (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining). The proportion and intensity were then summed to produce total scores of 0 or 2 through 8. A score of 0–2 was regarded as negative while 3–8 as positive.

HER2 immunohistochemical staining was scored according to the guidelines published by Ellis et al. [19]. Tumors that showed strong complete membrane staining in >10% of the tumor cells were considered positive.

### Statistical Analysis

Data were fed to the computer using the Predictive Analytics Software (PASW Statistics 18). Association between categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test. Parametric statistical tests were applied when either the sample size exceeded 30 or the variable distribution was not significantly different from normal. Independent *t*-test (parametric) or Mann–Whitney test (non-parametric) were used to compare quantitative variables between two groups.

Survival analysis was used to determine the relation between different clinical and laboratory factors and the recurrence or metastasis of the disease. Patients with follow up duration <6 months were excluded leaving 88 patients to be included in the survival analysis. Kaplan–Meier plot was used to estimate the survival function among different groups. Log-rank test was used to compare survival distributions among different samples. As regards studying the relation between the occurrence of the event and other quantitative variables univariate Cox regression was used. Both hazard rate and 95% CI were computed to determine the effect of quantitative variables on the occurrence of the event. This was followed by multivariate Cox regression analysis to identify independent predictors of survival of the studied breast carcinoma patients. Its application followed the enter method. The model as a whole was assessed using Model Chi-square. The contribution of different predictors was assessed using adjusted hazard rate and 95% CI. Life table was used to estimate 1, 2 and 3 year survival of the studied breast carcinoma patients.

## Results

### Clinicopathological Data

Our study population included 91 female patients with a median age of 49 years, mean 50.1±11.6 years (range from 27 to 76 years). Patients with follow up <6 months (n=4) were excluded from the survival analysis. The PFS (the mean time to event occurrence; whether recurrence and/or metastasis) was 36.68 ±1.54 months. Seventy three patients (80.2%) underwent tamoxifen treatment, 18.7% of cases were locally advanced at time of presentation and received preoperative neoadjuvant therapy while 19.8% of cases had a recurrence of breast carcinoma during follow-up.

### ER, PR, and HER-2 Status

All 91 patients were evaluated for ER, PR, and HER2 immunohistochemical expression; of whom 74.7% were ER positive (ER+), 69.2% were PR positive (PR+), 42.9% were HER2 positive (HER2+) and 12.1% were triple-negative (ER-/PR-/HER2-).

### Quantitation of Caveolin-1 (Cav-1) Immunostaining

All 91 patients were semiquantitatively scored separately for stromal and epithelial cav-1 expression. Cav-1 grading scale was done (0, 1, and 2), with (0) representing an absence of cav-1 and (2) representing high levels of cav-1. Scores of 1 and 2 were interpreted as cav-1 positive.

In the present study, the normal breast tissue adjacent to the tumor revealed cav-1 expression in MECs around breast ducts and lobules. Luminal epithelial cells were totally cav-1 negative. As regards the stromal compartment, the intralobular

fibroblasts (i.e. fibroblasts of the modified stroma) showed strong membranous staining for cav-1, whereas the interlobular and periductal fibroblasts were either negative or showed weak-to-moderate staining. Adipocytes, endothelial cells and perineurial cells showed consistent, strong cav-1 staining and served as internal positive controls. (Fig. 1a)

Absence of stromal caveolin-1 immunostaining (score 0) was detected in 18.7% (17/91) of studied cases, while 74 cases showed positive stromal caveolin-1 immunostaining. Out of these 74 positive cases, 30 cases (40.5%) were score 1, and 44 cases (59.5%) were score 2. (Fig. 1b, c)

To assess the predictive value of epithelial cav-1 expression, the same patient breast tumor samples were scored for cav-1 expression in the epithelial tumor cells, using the same scoring scheme as for stromal cav-1 (0=absent; 1 or 2= positive). Sixty eight (74.7%) cases were negative for tumor epithelial cav-1 staining, and only 23 cases (25.3%) showed caveolin-1 positive immunostaining in breast carcinoma cells. Of these 23 cases; 15 cases (16.5%) revealed weak cav-1 immunoreactivity (score 1), and 8 cases (8.8%) were strongly positive for caveolin-1 (score 2). (Fig. 1d, e)

Out of the 91 cases, 57 cases (62.6%) showed ductal carcinoma in situ (DCIS) areas. Caveolin-1 expression was detected as a continous or interrupted stain in MECs around involved ducts, and only a single case displayed positive caveolin-1 staining in DCIS tumor cells. (Fig. 2a, b, c, d,and e)

Combined stromal and epithelial tumor cell caveolin-1 immunopositivity was seen in 24.2% of the studied cases (n=22). (Fig. 2f)

## Caveolin-1 Expression Related to Pathological Features

In this study, the relationship between standard prognostic factors and stromal cav-1 expression is shown in Table 1, which reveals that absence of stromal Cav-1 was strongly associated with markers of more aggressive disease.

Caveolin-1 expression in breast tumor cells was also assessed in relation to factors known to be associated with tumor progression, and revealed a statistically significant relation only with triple negative status ($p$=.005).

## Cav-1 Expression Related to Survival

The association between stromal cav-1 expression and survival was assessed. Loss of stromal cav-1 was significantly associated with higher recurrence rate, and shorter mean PFS (PFS was 37.34 ± 1.37 months in stromal cav-1 positive vs 26.62 ± 4.47 months in stromal cav-1 negative cases, ($p$=.001). Patients with absent stromal cav-1 expression showed a four-fold and a two fold increase in disease recurrence rate during the first and second years of follow up respectively compared to stromal cav-1 positive patients.

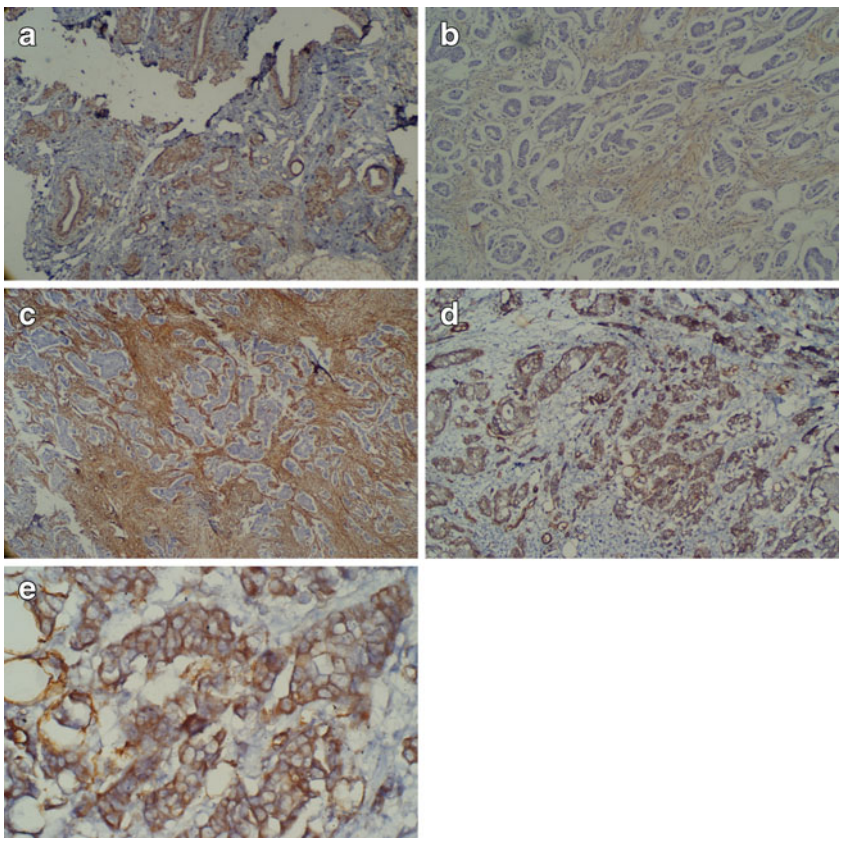


**Fig. 1** Caveolin −1 immunostaining. **a** Normal breast tissue demonstrating strong expression in ductal myoepithelial cells, vascular endothelial cells and adipocytes,(X100). **b** and **c** Infiltrating ductal carcinoma showing weak (**b**) and strong (**c**) expression in the stromal cells and negative reaction of breast carcinoma cells, (X100). **d** and **e** Infiltrating ductal carcinoma showing strong expression in breast carcinoma cells, (d, X100 and e, X 400)

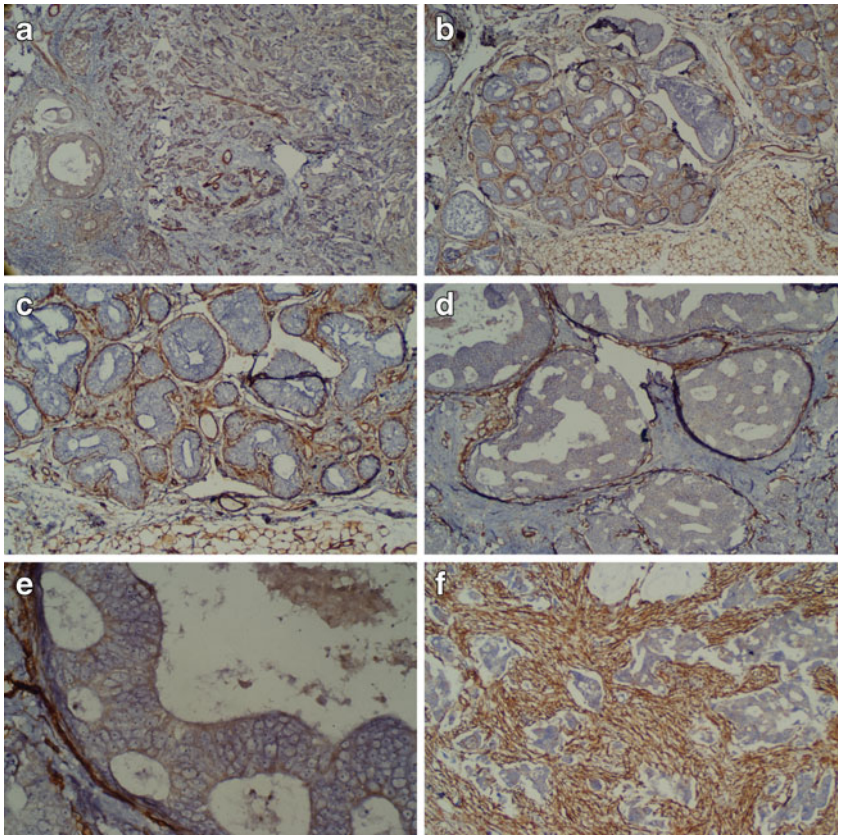


**Fig. 2** Caveolin-1 expression in breast carcinoma. **a** Both invasive and in situ ductal tumor cells stained positive for caveolin-1, with strong staining of vascular endothelial cells that were used as internal control), (X40). **b** and **c** Myoepithelial cells around ducts involved by insitu carcinoma and adipocytes stained strongly for caveolin-1; both served as internal positive control, (X100). **d** and **e** Weak caveolin-1 expression (score 1) in the in situ ductal tumor cells with strong staining of surrounding myoepithelial cells and vascular endothelial cells which served as internal positive control, (d, X100 and e, X400). **f** Combined strong stromal (score 2) and weak breast tumor cell (score 1) caveolin-1 expression, (X100)

The recurrence rate during the third year did not differ between stromal cav-1 negative and positive cases. The 3 year hazard rate in stromal cav-1 negative cases was 0.02 versus a hazard rate of 0.01 in stromal cav-1 positive cases. These results point to a significant role played by the absence of stromal cav-1 in the development of early tumor recurrence and thus shorter mean PFS. Thus, stromal cav-1 expression status is a strong predictor of clinical outcome in breast carcinoma patients. In opposition, epithelial cav-1 expression did not associate with patients' clinical outcome. This is an important internal control for our current study, and reinforces the idea that stromal cav-1 expression is a primary determinant of clinical outcome in breast cancer patients, (Fig. 3).

As absence of stromal cav-1 behaved as a predictor of disease recurrence and poor clinical outcome, we tried to assess the predictive role of absence of stromal cav-1 in LN negative versus LN positive patients. The predictive role of stromal cav-1 in LN negative cases could not be tested as all node negative patients were stromal cav-1 positive. Thus, we only tested the predictive role of stromal cav-1 in LN positive patients. Absence of stromal cav-1 proved to remain a significant predictor of progression- free outcome as PFS was shorter in stromal cav-1 negative compared to stromal cav-1 positive cases (26.62 ± 4.47 months versus 37.80 ± 1.49 months respectively); ($p$=.001).

Also, we examined the predictive role of absence of stromal cav-1 within a grade or within a stage. Absence of stromal cav-1 was significantly associated with earlier tumor recurrence and shorter PFS within tumor grades I and II ($p$=.049), and did not reach significance within grade III tumors. Similarly, when tested for stage, absence of stromal cav-1 was significantly associated with earlier tumor recurrence and shorter PFS within stage III tumors, ($p$= <.001), was nearly significant in stage IV tumors, ($p$=.052) but, was not tested in stages I and II as all cases were stromal cav-1 positive.

In addition, we tested if stromal cav-1 could act as a strong predictive biomarker independent of all three established epithelial markers (ER, PR, HER2), as seen in Kaplan Meier plots (Figs. 4, 5, and 6), that revealed that stromal cav-1 status serves as an important significant predictor of PFS in ER+, PR +, and HER2+.

Moreover, within the hormonally treated group (n=73, 80.2%), absence of stromal cav-1 was significantly associated with higher event rate and shorter mean PFS (27.18 ± 4.65 versus 38.53 ± 1.35), thereby, suggesting an association with underlying tamoxifen resistance, Fig. (7)

In the current study, we were not able to examine the predictive value of absence of stromal cav-1 in triple-negative patients, as all 10 triple negative cases included in the current study were stromal cav-1 positive. Also, testing the role of stromal caveolin-1 in promoting metastasis was

**Table 1** Relation between Stromal Caveolin-1 and Clinicopathologic parameters

| | Stromal Caveolin status | | Total | Test (p-value) |
|---|---|---|---|---|
| | Negative | Positive | | |
| Age | | | | t=.525 (.601) |
| Mean ± SD | 51.47 ± 12.61 | 49.82 ± 11.42 | 50.13 ± 11.60 | |
| Tumor size | | | | $X^2$=7.393 (.007) |
| T1,2 | 1(5.9) | 30(40.5) | 31(34.1) | |
| T3,4 | 16(94.1) | 44(59.5) | 60(65.9) | |
| Tumor grade | | | | FET (.028) |
| I,II | 9(52.9) | 58(80.6) | 67(75.3) | |
| III | 8(47.1) | 14(19.4) | 22(24.7) | |
| Tumor stage | | | | $X^2$=10.472 (.005) |
| I,II | 0 (0) | 27 (39.1) | 27 (31.8) | |
| III | 11 (68.8) | 34 (49.3) | 45 (52.9) | |
| IV | 5 (31.3) | 8 (11.6) | 13 (15.3) | |
| LN status | | | | FET (.114) |
| Negative | 0(0) | 10(15.2) | 10(12.0) | |
| Positive | 17(100.0) | 56(84.8) | 73(88.0) | |
| Nodal-stage | | | | $X^2$=24.934 (<.001) |
| N0 | 0(0) | 10(15.2) | 10(12.0) | |
| N1 | 2(11.8) | 30(45.5) | 32(38.6) | |
| N2 | 4(23.5) | 19(28.8) | 23(27.7) | |
| N3 | 11(68.8) | 7(10.6) | 18(21.7) | |
| Triple_negative status | | | | FET (.117) |
| ER-, PR-, HER2- | 0(0) | 11(14.9) | 11(12.1) | |
| Others | 17(100.0) | 63(85.1) | 80(87.9) | |
| ER status | | | | FET (1.000) |
| Negative | 4(23.5) | 19(25.7) | 23(25.3) | |
| Positive | 13(76.5) | 55(74.3) | 68(74.7) | |
| PR status | | | | $X^2$=.201 (.654) |
| Negative | 6(35.3) | 22(29.7) | 28(30.8) | |
| Positive | 11(64.7) | 52(70.3) | 63(69.2) | |
| HER2 status | | | | $X^2$=6.565 (.010) |
| Negative | 5(29.4) | 47(63.5) | 52(57.1) | |
| Positive | 12(70.6) | 27(36.5) | 39(42.9) | |
| Epithelial caveolin-1 status | | | | FET (.061) |
| Negative | 16(94.1) | 52(70.3) | 68(74.7) | |
| Positive | 1(5.9) | 22(29.7) | 23(25.3) | |
| Tamoxifen use | | | | FET (.508) |
| No | 2 (11.8) | 16 (21.6) | 18 (19.8) | |
| Yes | 15(88.2) | 58(78.4) | 73 (80.2) | |
| Number of positive LN | | | | Z=−4.094 (<.001) |
| Median(Min.- Max) | 10.00 (1–15) | 3.00(0–15) | 3(0–15) | |

limited as all metastatic cases at time of presentation experienced an event occurrence during follow up regardless of stromal cav-1 status.

A significant association was detected between combined (tumor cell and stromal) caveolin-1 immunopositivity and triple negative status ($p$=.003). However, when the predictive value of combined cav-1 immunostaining in those cases was tested; it was insignificant, ($X2$ Log rank test=.161, $p$=.688).

The relation between known breast carcinoma prognostic factors with survival on bivariate analysis is shown in Table 2. A model was developed to determine the independent predictors of progression of the disease among breast carcinoma patients. The predictive power of the model was significantly higher than that of the baseline model (Model $X2$=48.50, $p$<.001). Stage of the tumor (Wald statistic=5.79, $p$=.050) was found to be the only independent predictor. Controlling for all other character-

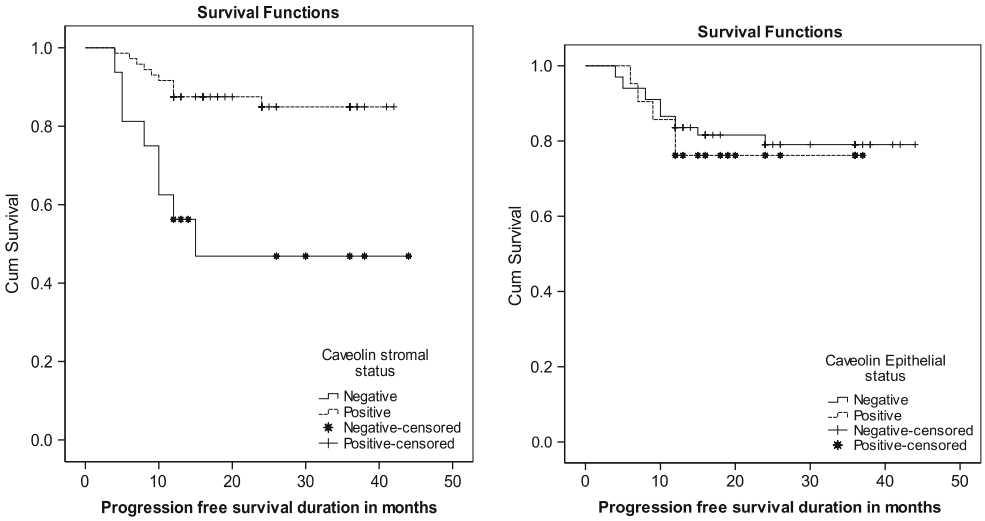


**Fig. 3** Kaplan Meier plots showing the predictive value of stromal and epithelial caveolin-1 status

istics included in the model, the estimated risk of disease progression was highest among patients with tumor stage IV (HR=13.76; 95% CI=2.65, 71.49, $p$=.002) in relation to patients with tumor staged as I, II.

Thus, our data point that the use of stromal Cav-1 as a predictive biomarker, especially in lower grade, lower stage, ER+, PR+, HER2+ and tamoxifen treated patients may allow for early interventions with more aggressive therapies.

## Discussion

The role of caveolin-1 in mammary carcinogenesis is not completely understood [20]. Discrepancies in literature regarding the proposed pro- or anti-neoplastic roles of caveolin-1 were noted in the studies that did not discriminate between stromal and tumor cav-1 expression [8].

Although epithelial cav-1 expression has been extensively studied in breast carcinomas, yet, little is known on the expression and significance of stromal cav-1 in breast carcinomas [3, 21–23]. This study was undertaken to evaluate the stromal and tumor epithelial cell expression of caveolin-1 in a cohort of breast carcinoma patients, and to assess the association between stromal cav-1 expression and clinicopathologic variables as well as patient outcome and early tumor recurrence.

In this study, consistent with others [17, 23, 24], the normal breast tissue adjacent to carcinoma areas revealed positive cav-1 immunoreactivity in the stromal fibroblasts, in MECs surrounding the normal breast ducts and lobules, as well as in adipocytes and vascular endothelial cells, whereas, luminal epithelial cells were consistently cav-1 negative, although, epithelial cav-1 expression was reported by others [17, 25, 26].

We applied a 3-tiered semiquantitative score to describe cav-1 expression, and based on the total absence of cav-1 expression in the normal breast epithelial cells, we combined weak and strong expression (scores 1 and 2) to

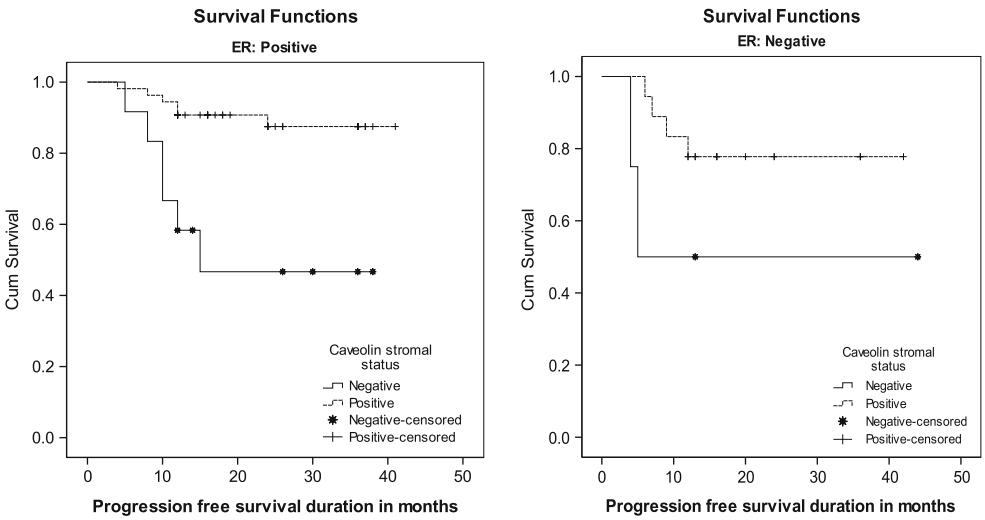


**Fig. 4** Kaplan-Meier plots showing the predictive value of stromal caveolin-1 in ER-positive versus ER negative patients

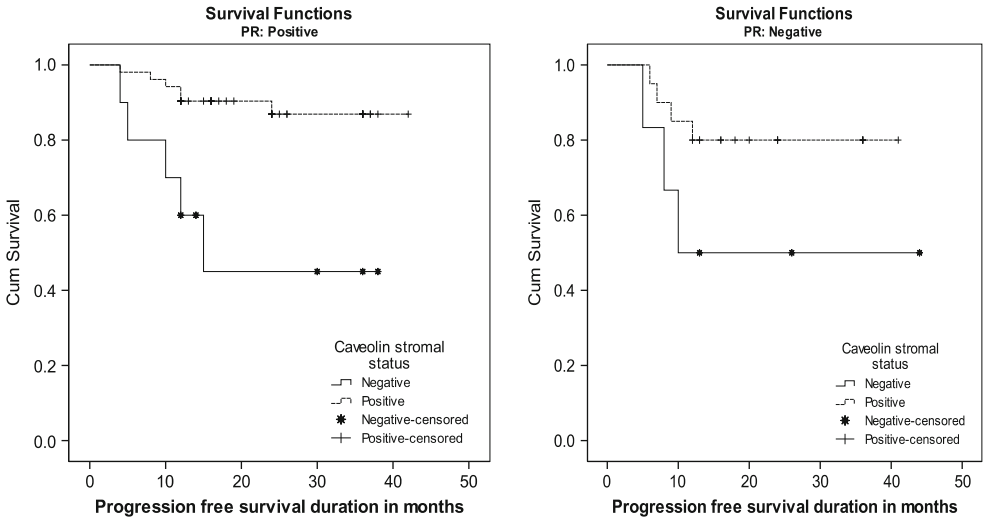


**Fig. 5** Kaplan-Meier plots showing the predictive value of stromal caveolin-1 in PR positive versus PR negative patients

represent cav-1 positive immunostaining. Accordingly, we observed positive cav-1 expression in the stromal cells in 81.3%, and in tumor cells in 25.3% of studied cases. Combined stromal and tumor cell positive cav-1 immunoreactivity was seen in 22 cases. Also, 62.6% of the studied cases showed DCIS areas that revealed cav-1 positive immunostaining in MECs surrounding the involved ducts, while DCIS tumor cells were cav-1 positive in only a single case.

Conversely, Yang et al. [27], using a polyclonal antibody reported minimal cav-1 expression in normal breast epithelium and positive cav-1 staining in 80% of their studied DCIS cases. However, Hurlstone et al. [24], using a monoclonal antibody, in accordance with our results, reported total negativity of cav-1 expression among normal breast luminal epithelial cells. Thus, choice of the primary antibody has a substantial impact on the results of immunoreactivity [1]. Also, variation in scoring methods might explain some of the discrepancies between our results and those of Savage et al. [17], who applied a semiquantitative consensus score of both distribution and intensity of cav-1 staining, and based on a cutoff score of ≥4 they reported cav-1 expression in 9.4% of primary breast cancers, and in 13.4% of DCIS tumor cells [3].

Many reports linked caveolin-1 to tumor progression and clinical outcome in different types of cancer, but without defining its role as a prognostic marker [8]. In accordance with others [3, 8], we report that absence of stromal cav-1 expression associates significantly with higher tumor grade and stage at time of diagnosis. Also in agreement with Witkiewicz Ak et al. [3], our data showed that loss of stromal cav-1 associated significantly with larger tumor size, higher nodal stage and greater number of involved nodes. In bivariate survival analysis absence of stromal cav-1 expression, associated with increased recurrence rate and shorter PFS, however, it did not prove to be an independent prognostic factor in multivariate survival analysis. All of

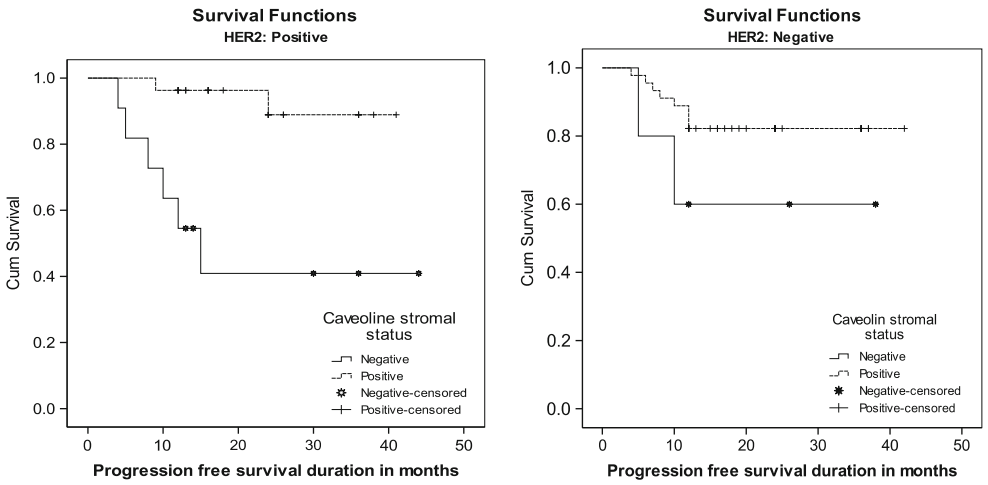


**Fig. 6** Kaplan-Meier plots showing the predictive value of stromal caveolin-1 in HER-2 positive versus HER-2 negative patients

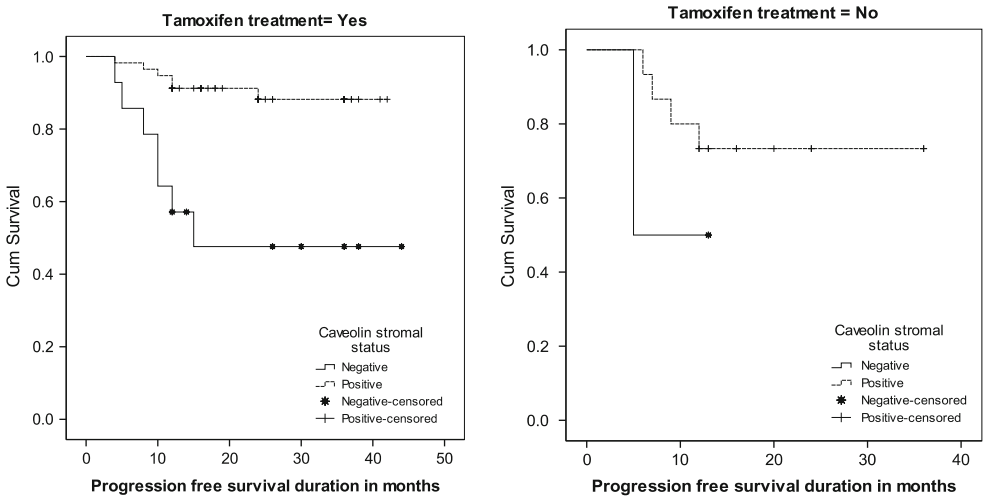


**Fig. 7** Kaplan-Meier plots showing the predictive value of stromal caveolin-1 in patients with and without tamoxifen treatment

these findings suggest that the presence of caveolin-1 positive stromal elements in the primary tumor microenvironment is associated with improved outcome in breast carcinoma. Conversely, tumor epithelial cell cav-1 positive staining did not associate with survival and patient outcome. Thus, our findings confirm previous reports [3, 8], that the loss of stomal cav-1 expression has a very strong correlation with poorer clinical outcome while expression within the tumor epithelium is not predictive of outcome.

Similarly, Savage K et al. [17], reported that cav-1 expression significantly associated with shorter overall survival but did not prove to be an independent prognostic factor in multivariate survival analysis, however others [3, 8] reported stromal caveolin-1 status to be a reliable and powerful single marker to predict breast carcinoma recurrence independent of standard clinicopathological risk factors and treatment regimens.

In agreement with others [8, 25, 28], loss of stromal cav-1 significantly associated with positive HER2 satus, but not with hormone receptors. Still yet, similar to Witkiewicz Ak et al. [3] within the hormonally treated group, absence of stromal cav-1 significantly associated with higher recurrence rate and shorter PFS thereby, suggesting an association with underlying tamoxifen resistence.

As ER, PR, and HER2 expression are biomarkers for stratifying breast cancer patients into different diagnostic and therapeutic groups, we assessed stromal cav-1 status in the different patient groups within our cohort. We observed that absence of stromal cav-1 predicts also early tumor recurrence and poor clinical outcome in ER+, PR+, and HER2+ tumors, thereby highlighting that the tumor stroma may be a primary determinant of disease recurrence and poor clinical outcome in breast carcinoma patients. Thus, tumor stroma should be more actively targeted in breast cancer therapeutic interventions. Conversely, Witkiewicz Ak et al. [3], reported that regardless of epithelial marker status for ER, PR, or HER2, stromal cav-1 serves as an important predictor of progression-free outcome, and may serve as a new predictive biomarker.

In our study, triple negative status associated significantly with combined cav-1 positive staining, and based on the consistent strong expression of cav-1 in the MECs of the normal breast duct and lobules and in MEC surrounding breast ducts involved by DCIS, we suggest that triple negative cases that showed combined epithelial tumor cell and stromal cav-1 expression may include subsets of breast carcinoma with basal-like phenotype, which needs further confirmation by immunostains for basal markers. This goes with Savage et al. [7], who reported that 70% of infiltrating ductal carcinoma with basal-like phenotype showed cav-1 immunopositivity.

Testing the role of stromal caveolin-1 in promoting metastasis was limited in this study as all cases that were metastatic at time of presentation experienced an event occurrence during follow up regardless of stromal cav-1 status. Thus, future studies are recommended to verify any role for loss of stromal cav-1 in promoting distant metastasis, as previous reports suggested stromal caveolin-1, either at the primary site or potentially in distant tissues, may provide a tumor suppressor influence that inhibits growth of metastatic nodules [8]. This suggestion was based on cav-1 strong expression in the normal breast MECs that was linked to the tumor suppressive function of these cells [5].

Loss of stromal caveolin-1 is a novel breast cancer biomarker that can predict early disease recurrence, poorer progression free survival, and tamoxifen-resistance, thereby, highlighting that stromal marker expression and epithelial-stromal cross talk may be critical for tumor

**Table 2** Predictors of progression of breast Carcinoma (Bivariate and multivariate analysis)

| | No. | Events No. (%) | Mean ± S.E | Log rank test $X^2$ (p-value) | Adjusted HR(95% CI) (p-value) |
|---|---|---|---|---|---|
| Tumor size (T-stage) | | | | 3.043 (.081) | |
| T1,2 | 30 | 3 (10) | 38.73±1.79 | | |
| T3,4 | 58 | 15 (25.8) | 34.62±2.08 | | |
| Tumor grade | | | | 4.897 (.027) | |
| I,II$^R$ | 64 | 10 (15.6) | 38.49±1.60 | | |
| III | 22 | 8 (36.3) | 25.39±3.02 | | 1.75 (.67–4.59), p=.254 |
| Tumor stage | | | | 47.696 (<.001) | |
| I,II$^R$ | 27 | 2 (7.4) | 36.00±1.35 | | |
| III | 44 | 5 (11.3) | 39.81±1.76 | | 1.47 (.28–7.76), p=.653 |
| IV | 13 | 11 (84.6) | 13.46±2.77 | | 13.76(2.65–71.49),p=.002 |
| Nodal-stage | | | | 3.825 (.281) | |
| N0 | 10 | 1 (10) | 33.75±2.81 | | |
| N1 | 31 | 6 (19.3) | 32.51±2.01 | | |
| N2 | 23 | 3 (13.0) | 33.17±2.07 | | |
| N3 | 17 | 6 (35.2) | 31.36±4.15 | | |
| *LN status* | | | | .685 (.408) | |
| Negative | 10 | 1 (10) | 33.75±2.8 | | |
| Positive | 71 | 15 (21.1) | 36.48±1.72 | | |
| Combined epithelial and stromal Caveolin −1 | | | | .322 (.570) | |
| No | 68 | 13 (19.1) | 37.08±1.72 | | |
| Double positive | 20 | 5 (25) | 30.05±2.70 | | |
| ER status | | | | 1.251 (.263) | |
| Negative | 22 | 6 (27.2) | 33.95±3.51 | | |
| Positive | 66 | 12 (18.1) | 35.22±1.51 | | |
| PR status | | | | 1.155 (.283) | |
| Negative | 26 | 7 (26.9) | 34.34±3.12 | | |
| Positive | 62 | 11 (17.7) | 36.06±1.62 | | |
| HER2 status | | | | .013 (.908) | |
| Negative | 50 | 10 (20) | 35.32±1.89 | | |
| Positive | 38 | 8 (21.05) | 36.24±2.43 | | |
| Stromal caveolin-1 | | | | 12.398 (.001) | |
| Negative$^R$ | 16 | 8 (50) | 25.40±4.64 | | |
| Positive | 72 | 10 (13.8) | 37.39±1.35 | | .721 (.254- 2.047),p=.539 |
| Epithelial caveolin-1 | | | | .200 (.655) | |
| Negative | 67 | 13 (19.4) | 37.00±1.74 | | |
| Positive | 21 | 5 (23.8) | 30.38±2.59 | | |
| Tamoxifen Treated | | | | 1.481 (.224) | |
| No | 17 | 5 (29.4) | 27.70±3.13 | | |
| Yes | 71 | 13 (18.3) | 37.53±1.62 | | |
| Triple negative status (ER-,PR-,HER2-) | 10 | 3 (30) | 27.70±4.03 | .767 (.381) | |
| Others | 78 | 15 (19.2) | 37.12±1.59 | | |

$^R$ refers to reference group

progression and metastasis. We recommend an extended study on a larger number of triple negative patients with the use of basal markers to show if combined tumor cell and stromal cav-1 positivity identifies breast carcinoma cases of basal-like/myoepithelial phenotype. Also, we suggest that evaluating stromal caveolin-1 status at time of initial diagnosis may be an effective prognostic factor that would allow individualization of patient therapy. Thus, further prospective clinical trials to confirm the prognostic power of stromal caveolin-1 in primary breast carcinoma are warranted.

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