RESEARCH ARTICLE

Editorial Process: Submission:04/06/2024 Acceptance:07/18/2024

Immunohistochemical Expression of *Caspase1* and Epidermal Growth Factor Receptor in Invasive Breast Carcinoma and Their Biological and Prognostic Associations

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Abstract

Background: Despite advances in breast carcinoma therapies, drug resistance mechanisms as anti-apoptosis and anti-pyroptosis limit the application of these therapies. This work assesses the immunohistochemical (IHC) expression of *Caspase1* and *EGFR* in breast carcinoma and analyzes their clinicopathological associations as prognostic markers and potential therapeutic targets. *Caspase1/EGFR* expression patterns are utilized to specify breast carcinoma patients who may benefit from these therapies. **Methods:** After reviewing the hematoxylin and eosin-stained slides and the routine breast carcinoma IHC stains (estrogen receptors, progesterone receptors, *HER2*/NEU, Ki-67) by two pathologists and preparation of tissue microarray blocks, anti-*Caspase-1* and *EGFR* IHC staining was performed using Horseradish Peroxidase (HRP) technique. Intensity and percentage-based scoring was applied dividing the 153 included breast carcinomas into *Caspase1*-negative and positive expression groups; and *EGFR* low and overexpression groups. Groups were statistically analyzed in relation to age, tumor size, histological and molecular subtype, grade, nodal status, metastasis/recurrence, TNM stage and Ki-67 proliferation index. Kaplan-Meier's analysis was used to compare disease-free survival (DFS) and overall survival (OS). Combined patterns based on *Caspase1* and *EGFR* expression status were created to stratify patients into prognostic groups. **Results:** *Caspase1* was positive in 54.2% of breast carcinomas and its positivity was significantly associated with smaller tumor size, absence of metastasis/recurrence, luminal A and B molecular subtypes and longer OS ($p<0.05$). *EGFR* overexpression was detected in 32.7% of carcinomas and was significantly associated with larger tumor size, TNBLBC and a shorter OS ($p<0.05$). *Caspase1*-negative/*EGFR*-overexpression pattern comprised 14.4% of carcinomas and had the worst prognostic associations including larger tumor size, metastasis/recurrence, TNBLBC subtype and shortest OS ($p=0.002$, 0.002, 0.004 and ≤ 0.001 respectively). **Conclusions:** Combined *Caspase1*/*EGFR* IHC expression may provide a tool for selection of patients who benefit from combined *EGFR*-inhibitors with miR-155-5p down-regulators or photodynamic therapy via induction of apoptosis/pyroptosis in *EGFR*-overexpression carcinomas through enhanced *Caspase1* signaling.

Keywords: *Caspase1- EGFR-* immunohistochemistry- pyroptosis- apoptosis- prognosis

Asian Pac J Cancer Prev, **25 (7)**, 2529-2537

Introduction

Breast cancer is the most common malignancy in women and is the most frequently diagnosed cancer in the vast majority of countries. Based on the GLOBOCAN's 2020 estimates of incidence and mortality for 36 cancer types in 185 countries, breast cancer represented 11.7% of newly diagnosed cancer cases and 6.9% of reported cancer deaths [1]. Despite the potential efficacy of immunotherapy, particularly anti-Programmed cell death protein 1 (anti-PD-1), and the epidermal growth factor receptor (*EGFR*) antibodies/inhibitors in treating triple-negative breast cancer (TNBC), the emergence of multiple drug resistance mechanisms, including anti-apoptosis and anti-pyroptosis in tumor cells, has considerably impeded the clinical application of these therapies [2, 3]. It is, therefore, necessary to conduct more in-depth research to identify more effective therapeutic options for breast cancer [4, 5].

Pyroptosis is a type of cell death that depends on *Caspase1* activation. Emerging evidence indicates that pyroptotic cell death leads to suppression of tumor growth. The identification of markers that trigger pyroptosis in breast cancer cells serves as a foundation for developing pyroptosis-targeting strategies in breast cancer [6]. *Caspase1* may be a novel target molecule for treating

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breast cancer [7, 8]. Yet, there are few studies on the expression characteristics and significance of pyroptosis in solid tumors, notably in breast cancer tissues and most of these studies was conducted on cancer cell lines or on a few numbers of breast cancer patients [4, 9].

Another newly-developed molecular target in breast cancer is the *EGFR* encoded by a gene located on chromosome 7p11.2. *EGFR* is a receptor tyrosine kinase that belongs to the ErbB family and functions as an oncogene involved in angiogenesis, cell proliferation, metastases as well as inhibition of apoptosis. It has been shown to be an independent prognostic indicator of worse disease-free survival (DFS) and overall survival (OS), especially in triple-negative breast cancer (TNBC) [10-12]. Therefore, the evaluation of *EGFR* overexpression should be performed in to identify patients who may benefit from anti-*EGFR*-targeted therapy [13].

Photodynamic therapy, a minimally invasive cancer therapy, has been found to be a successful anti-tumor strategy in preliminary clinical trials, particularly for breast cancer treatment. Recently, a combination of *EGFR* Inhibitors and photodynamic therapy was found to suppress breast cancer progression and enhance cancer cell apoptosis and pyroptosis via a mechanism involving a significant increase in *Caspase1* [5, 14]. Moreover, the downregulation of microRNA-155-5p was found to enhance the anti- tumor effect of *EGFR*-inhibitors as cetuximab on TNBC cells via inducing apoptosis and pyroptosis [2]. Therefore, an interplay between *EGFR* and *Caspase1* in breast cancer may exist and a study of combined *EGFR* and *Caspase1* expression in breast carcinoma may provide more insights in this field.

This work aims to assess the frequency of *Caspase1* and *EGFR* expression in breast carcinoma using immunohistochemistry (IHC), and to describe the clinicopathological features associated with different *Caspase1* and *EGFR* expression patterns as prognostic, predictive markers, and potential therapeutic targets in breast cancer. *Caspase1* and *EGFR* are combined in a prognostic model to stratify breast cancer patients.

Materials and Methods

Patients and Methods

This retrospective cohort study was conducted at the Pathology Laboratory, Oncology Center, Mansoura University (OCMU), Faculty of Medicine, Mansoura University, Egypt. Paraffin-embedded tissue blocks were obtained from the archived lab material during the period from January 2014 to June 2018 to ensure a follow-up period of at least 36 months.

Subjects, Inclusion and Exclusion Criteria

Patients were selected via electronic database search for cases diagnosed with invasive breast carcinoma during the specified study period. Patients who didn't receive preoperative adjuvant chemo/radiotherapy, whose archived formalin-fixed, paraffin-embedded (FFPE) tumor tissue blocks were available for further study and whose follow-up data were available through regular clinical visit-records were enrolled. Cases not fulfilling any of the abovementioned criteria were excluded from the study.

Data Collection

The following data were abstracted from medical, surgical and pathological records: patient's age, gender, tumor size (T), presence of clinically/radiologically or pathologically confirmed nodal (N) or distant metastases (M), and the duration of patient's survival in months; with the disease-free survival (DFS) period calculated starting from the date of initial diagnosis till the end of study period (by conduction of statistical analysis) or the detection of recurrence/metastasis (or re-appearance of any signs or symptoms of cancer) and the duration of overall survival (OS) calculated from the initial diagnosis to death or the end of study period.

Histopathologic Evaluation

The FFPE tissue blocks and the hematoxylin and eosin (H&E)-stained microscopic slides were retrieved from the OCMU Pathology Laboratory archives. H&E slides were reviewed by two pathologists for: confirmation of the diagnosis, histological subtyping of breast carcinoma according to the World Health Organization (WHO) classification of breast neoplasms [15], grading of tumors according to Elston/Nottingham modification of Bloom-Richardson grading system [16], and staging according to TNM staging system [17]. Evaluation of routine prognostic breast markers and proliferation index was performed based on re-examination of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor2 (HER2/NEU) to set the molecular subtype and estimate the Ki67 proliferation index of the included breast carcinomas.

Immunohistochemical (IHC) Staining

Tissue microarray (TMA) blocks were constructed using Manual Tissue Arrayer (MTA-1, cat.no.MP06, 0.6mm punch-size, Estigen Tissue Science, Estonia). Slides were stained using anti-*Caspase1* antibody (Servicebio, GB11383,1:500 dilution) and anti-*EGFR* antibody (RM0089RTU7, Medaysis). As per data sheet instructions, slides were pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20-30 min. in microwave followed by treatment with 3% hydrogen peroxide for 10 min., then incubated with the primary IHC antibodies for 1 hour at room temperature. Detection of immunoreactivity was done using goat anti-rabbit IgG (Horseradish- peroxidase; HRP) as a secondary antibody, diaminobenzidine (DAB) as the chromogen (Sakura USA, Poly HRP DAB kit; Cat No. 54-0117), and hematoxylin for counterstaining.

Immunohistochemical (IHC) Scoring
Caspase1 Scoring

Cytoplasmic staining was considered positive [4]. The staining score included two aspects: the staining intensity and the proportion of positive cells. The cells not stained or very lightly stained were scored as 0, the cells-stained light yellow were scored as 1, the cells stained brownish-yellow were scored as 2, and the cells-stained dark brown were scored as 3. The number of positive cells <5% was

scored as 0, the number of positive cells from 6% to 25% was scored as 1, the number of positive cells from 26% to 50% was scored as 2, the number of positive cells from 51% to 75% were scored as 3, and the number of positive cells ≥76% were scored as 4. The two scores were added together. A total score ≤3 was considered *Caspase1*-negative, and a score >3 was considered *Caspase1*-positive [18].

EGFR Scoring

EGFR expression was scored as follows: 0, no staining or weak membranous staining in <10% of the tumor cells; 1+, weak membranous staining in ≥10% of the tumor cells; 2+, moderate membranous staining in ≥10% of the tumor cells; 3+, strong membranous staining in ≥10% of the tumor cells. Complete and incomplete membranous staining were both accepted and the scores 2+ and 3+ were considered to be *EGFR*-overexpression, while the scores 0 and 1+ were considered to be *EGFR* low-expression [10].

Ethical Considerations

This retrospective cross-sectional, histopathological study was conducted after obtaining ethical approval from the committed IRB at Mansoura University (Code Number: R.23.09.2337). It was performed on tissue sections obtained from paraffin-embedded tissue blocks archived at Pathology Laboratory in OCMU, while maintaining the archived tissue material. The code numbers of paraffin blocks were used instead of patients' personal data to ensure confidentiality. The study procedure has not influenced any previous biopsy procedure or therapeutic decision. No further medical interventions were applied to patients as a part of the study procedure. All procedures were done in accordance with the current revision of Helsinki Declaration [19].

Statistical Analysis

The Statistical Package of Social Science (SPSS) program (Standard version 25) was used for data entry and analysis. Data normality was tested with one-sample Kolmogorov-Smirnov test. Data were presented as numbers, percentages, ranges (minimum and maximum) and/or mean ± standard deviation (SD) whenever appropriate. The association between *Caspase1*, *EGFR* expression and the clinicopathological variables was analyzed using Chi-square test, Monte Carlo test and independent sample-T test as appropriate. Kaplan-Meier survival analysis was run to plot curves and evaluate the DFS and OS differences using Log-Rank test. A statistically significant difference is accepted at a p-value of ≤0.05 and the level of significance is assumed to be higher whenever p-value is lower.

Results

Descriptive Data

As seen in Table 1, the study included 153 breast carcinoma patients. All patients were females ranging in age from 27 to 93 years (mean± standard deviation=58.3± 11.8 years). Invasive ductal carcinomas comprised 133 cases (86.9%), and the remainder 20 cases were 11 lobular, 4 mucinous, 2 medullary, 2 micropapillary and one papillary carcinoma/s. Most of the tumors were grade 2 carcinomas (68.6%). About 69.9% of carcinomas measured 2 cm or less in size (T1), 75.8% were associated with metastatic deposits in 3 or less lymph nodes (N1), and 33.3% were associated with distant metastases or recurrences. About 54.2% were presented at WHO stage III/IV. Concerning molecular subtypes, luminal A was the most frequent subtype (33.8%), followed by luminal B (23.4%) then the triple negative basal-like breast carcinoma (TNBLBC) that comprised 19.3% of cases. The median Ki67 proliferation index was 10% with a range from 2 to 60%.

Caspase1 and EGFR Expression Frequency and Association with the Prognostic Variables

Based on the defined score combining the staining

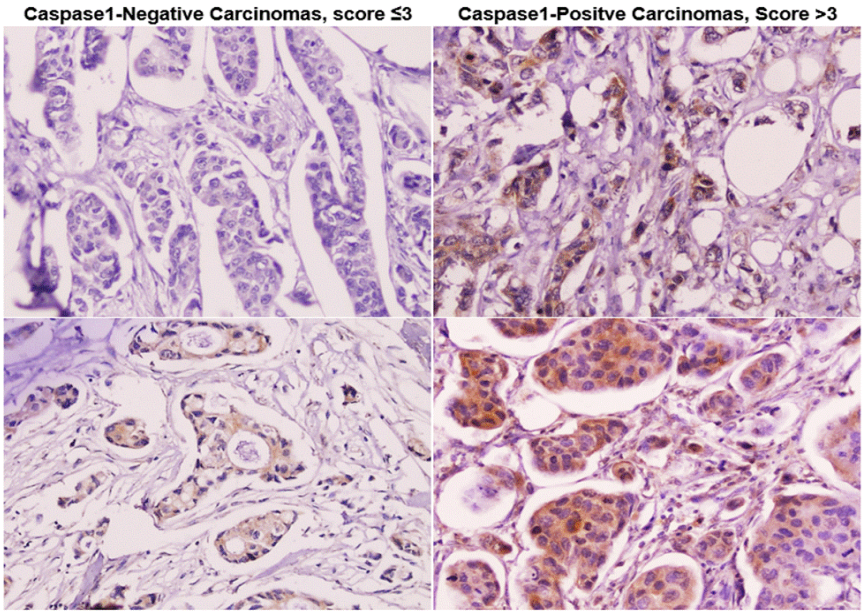

Figure 1. *Caspase1* Immunohistochemical Expression in Breast Carcinoma (Diaminobenzidine, x20).

Table 1. Descriptive Data, Caspase1 and *EGFR* Expression and Association with the Prognostic Variables

Variables	Total n=153	Caspase1 Expression		χ2 (p value)	EGFR Expression		χ2 (p value)
		Negative n=70, 45.8%	Positive n=83, 54.2%		Low expression n=103, 67.3%	Over expression n=50, 32.7%	
Age (years)				0.003 (0.9)			0.2 (0.6)
≤50	39 (25.5)	18 (25.7)	21(25.3)		25(24.3)	14(28)	
>50	114 (74.5)	52(74.3)	62(74.7)		78 (75.7)	36 (72)	
Histological Subtype				0.1(0.6)			0.07 (0.7)
Ductal	133 (86.9)	60 (87)	73 (89)		89 (86.4)	44 (88.0)	
Lobular/others	20 (13.1)	10 (13)	10 (11)		14 (13.6)	6 (12.0)	
Grade				1.3 (0.872)			5.6 (0.099)
N/A	20 (13.1)	10 (14.3)	10 (12)		1 (1)	0 (0)	
G1	1 (0.7)	0 (0)	1 (1.2)		14 (13.6)	6 (12)	
G2	105 (68.6)	49 (70)	56 (67.5)		75 (72.8)	30 (60)	
G3	27 (17.6)	11 (15.7)	16 (19.3)		13 (12.6)	14 (28)	
Tumor size in cm				6.06 (0.014*)			8.42 (0.004*)
≤2 (T1)	107 (69.9)	43(61.4)	66 (79.5)		81 (78.6)	28 (56.0)	
> 2 (T2,3,4)	46 (30.1)	27 (38.6)	17 (20.5)		22 (21.4)	22 (44.0)	
Lymph Node Status				3.5 (0.06)			0.7 (0.4)
N0/N1	116 (75.8)	58(82.9)	58 (69.9)		76(73.8)	40 (80)	
N2/3	37 (27.4)	12 (17.1)	25 (30.1)		27(26.2)	10 (20)	
Metastasis/recurrence				5.3 (0.022*)			0.37 (0.54)
No	102 (66.7)	40 (57.1)	62 (74.7)		67(65.0)	35 (70.0)	
Yes	51 (33.3)	30 (42.9)	21 (25.3)		36 (35.0)	15 (30.0)	
Stage				8.212 (0.101)			5.075 (0.383)
I, II	70 (45.8)	35 (58.3)	35 (42.2)		44 (42.7)	26 (52)	
III, IV	83 (54.2)	25 (41.7)	48 (57.8)		59 (57.3)	24 (48)	
ER				0.6 (0.4)			0.7 (0.3)
Positive	99 (64.7)	43 (61.4)	56 (67.5)		69 (67)	30 (60)	
Negative	54 (35.3)	27 (38.6)	27 (32.5)		34 (33)	20 (40)	
PR				0.5 (0.4)			2.03 (0.1)
Positive	86 (56.2)	37 (52.9)	49 (59)		62 (60.2)	24 (48)	
Negative	67(43.8)	33 (47.1)	34 (41)		41(39.8)	26 (52)	
HER2/NEU				1.2 (0.5)			0.6 (0.7)
Positive	29 (19.0)	13(18.6)	16(17.3)		19 (18.4)84 (81.6)	10 (20)	
Negative	124 (81.0)	57(81.4)	67(80.7)			40 (80)	
Molecular type				11.2 (0.021*)			11.9 (0.018*)
Luminal A	50 (33.8)	19 (27.1)	31(37.3)		39 (37.9)	11 (22.0)	
Luminal B	33 (23.4)	12 (17.1)	21 (25.3)		23 (22.3)	10 (20.0)	
HER2 Luminal	10 (6.9)	3 (4.3)	7 (8.4)		7 (6.8)	3 (6.0)	
TNBLBC	36 (19.3)	25 (35.7)	11 (13.3)		16 (15.5)	20 (40.0)	
HER2 Enriched	24 (16.6)	11 (15.7)	13 (15.7)		18 (17.5)	6 (12.0)	
Ki6 7 index	10	10	10	Z=1.4	10	10	Z=1.5 (0.1)
Median (min-max)	(2-60)	(2-60)	(2-60)	0.1	(2-60)	(5-60)	

EGFR, Epidermal Growth Factor Receptor; n, number; ER, Estrogen Receptor; PR, Progesterone Receptor; HER2/NEU, Human epidermal growth factor receptor2; TNBLBC, Triple-negative basal like breast cancer; min, minimum; max, maximum; χ2, Chi-square test, *p value is significant if ≤0.05.

intensity and the percentage of positive cells, 83 (54.2%) breast carcinomas showed a positive cytoplasmic staining for *Caspase1* (Figure 1), and 50 (32.7%) carcinomas overexpressed membranous *EGFR* (Figure 2, Table 1). *Caspase1* expression was significantly associated with smaller tumor size (as 79.5% of *Caspase1*-positive carcinomas were T1 compared to 61.4% of *Caspase1*-negative carcinomas; p=0.014), absence of metastasis/recurrence (as 74.7% of *Caspase1*-positive carcinomas were negative for metastasis or recurrence compared to 57.1% of *Caspase1*-negative carcinomas; p=0.022), and with the molecular subtype of carcinomas (as 37.3% of *Caspase1*-positive carcinomas were luminal A, while 35.7% of *Caspase1*-negative carcinomas were TNBLBC;

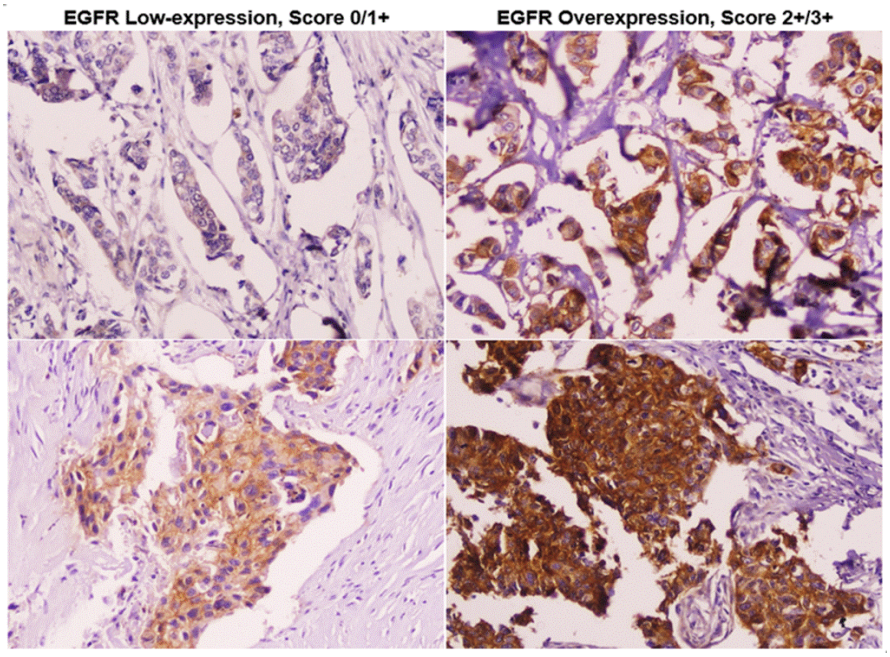

Figure 2. Epidermal Growth Factor Receptor (EGFR) Immunohistochemical Expression in Breast Carcinoma (Diaminobenzidine, x20).

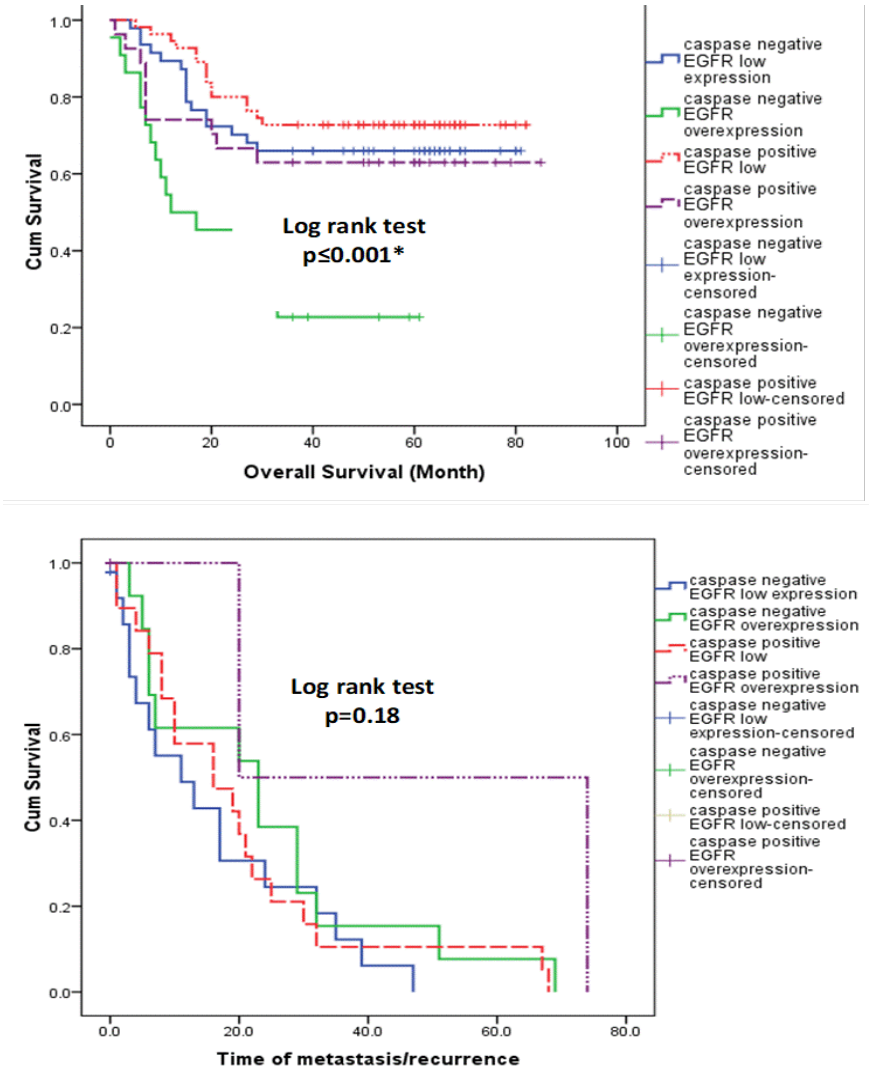

Figure 3. Kaplan-Meier Curves for Overall Survival (OS, Upper Panel) and Disease-Free Survival (DFS, Lower Panel) Compared among Different *Caspase1/EGFR* Expression Patterns in Breast Carcinoma Patients. *p value is significant if ≤0.05.

Table 2. Combined Caspase1 and *EGFR* Expression Patterns in Association with the Prognostic Variables

Variables	Caspase1-negative/ EGFR-low expression n=48, 31.4%	Caspase1- negative/ EGFR- overexpression n=22, 14.4%	Caspase1-positive /EGFR-low expression n=55, 35.9%	Caspase1-positive/ EGFR-overexpression n=28, 18.3%	χ2 (p value)
Age (years)					1.2 (0.75)
≤50	13 (27.1)	5 (22.7)	12 (21.8)	9 (32.1)	
>50	35 (72.9)	17 (77.3)	43 (78.2)	19 (67.9)	
Histological Subtype					1.4 (0.6)
Ductal	42 (87.5)	18 (81.8)	47 (85.5)	26 (92.2)	
Lobular/others	6 (12.5)	4 (18.2)	8 (14.5)	2 (7.1)	
Tumor size in cm					14.8 (0.002*)
≤2 (T1)	33 (68.8)	10 (45.5)	48 (87.3)	18 (64.3)	
> 2 (T2,3,4)	15 (31.2)	12 (54.5)	7 (12.7)	10 (35.7)	
Metastasis/recurrence					15.3 (0.002*)
No	31 (64.6)	9 (40.9)	36 (65.5)	26 (92.9)	
Yes	17 (35.4)	13 (59.1)	19 (34.5)	2 (7.1)	
ER					1.5 (0.6)
Positive	18 (37.5)	9 (40.9)	16 (29.1)	11 (39.3)	
Negative	30 (62.5)	13 (59.1)	39 (70.9)	17 (60.7)	
PR					3.4 (0.3)
Positive	22 (45.8)	11 (50)	19 (34.5)	15 (53.6)	
Negative	26 (54.2)	11 (50)	36 (65.5)	13 (46.4)	
HER2/NEU					14.1(0.9)
Positive	43 (89.6)	14 (63.6)	41 (74.5)	26 (92.9)	
Negative	5 (10.4)	8 (36.4)	14 (25.5)	2(7.1)	
Molecular Subtype					28.8 (0.004*)
Luminal A	18 (37.5)	1 (4.5)	21 (38.2)	10 (35.7)	
Luminal B	9 (18.8)	3 (13.6)	14 (25.5)	7 (25.0)	
HER2luminal	2 (4.2)	1(4.5)	5 (9.1)	2 (7.1)	
TNBLBC	12 (25.0)	13 (59.1)	4 (7.3)	7 (25.0)	
HER2 enriched	7 (14.6)	4 (18.2)	11 (20.0)	2 (7.1)	
Ki67 index					4.6 (0.1)
Median (min-max)	10 (2-60)	10 (5-50)	10 (2-40)	10 (5-60)	

EGFR, epidermal growth factor receptor; n, number; ER, estrogen receptor; PR, progesterone receptor; HER2/NEU, Human epidermal growth factor receptor2; TNBLBC, Triple-negative basal like breast cancer; min, minimum; max, maximum; χ2, Chi-square test, *p value is significant if ≤0.05.

Table 3. Overall Survival (OS) and Disease-free Survival (DFS) Analysis for Caspase1, *EGFR,* and Combined Caspase1 and *EGFR* Expression Patterns.

	OAS Mean (SE)	Log rank test p value	DFS Median (SE)	Log rank test P value
Caspase1		0.024*		0.7
Negative	49.2 (4.1)		16 (5.8)	
Positive	63.8 (3.5)		20 (4.7)	
EGFR		0.001*		0.3
Low-expression	62.2 (2.9)		20 (7.1)	
Overexpression	45.2 (5.2)		16 (3.08)	
Combined expression patterns		≤0.001*		0.18
Caspase1-negative/*EGFR*-low expression	58.5(4.5)		15 (3.6)	
Caspase1-negative/ *EGFR*-overexpression	24.6 (4.6)		23.3 (5.4)	
Caspase1-positive/ *EGFR*-low expression	64.7(3.8)		20.2 (4.5)	
Caspase1-positive/*EGFR*-overexpression	57.5 (6.9)		47.0 (27)	

EGFR, epidermal growth factor receptor; OS, Overall Survival; DFS, Disease-free Survival; SE, standard error, *p value is significant if ≤0.05.

p=0.021). *EGFR* expression was significantly associated with larger tumor size (as 44% of *EGFR* overexpression carcinomas were larger than 2cm compared to 21.4% of *EGFR* low-expression carcinomas; p=0.004), and with the molecular subtype of carcinoma (as 40% of *EGFR* overexpression carcinomas were TNBLBC, while 37.9 of *EGFR* low-expression carcinomas were luminal A; p=0.018).

Combined Caspase1 and EGFR Expression in Association with the Prognostic Variables

Combined *Caspase1*/*EGFR* expression patterns (Table 2) revealed that 35.9% of carcinomas are *Caspase1*-positive/*EGFR*-low expression, 31.4% are *Caspase1*-negative/*EGFR*-low expression, 18.3% are *Caspase1*-positive/*EGFR*-overexpression, and 14.4% of carcinomas are *Caspase1*-negative/*EGFR*-overexpression. There were significant statistical differences between different combined *Caspase1*/*EGFR* Expression patterns and the following prognostic variables: tumor size, metastasis/recurrence, and the molecular subtype of breast carcinoma (p=0.002, 0.002 and 0.004 respectively). Most of the *Caspase1*-positive/*EGFR*-low expression carcinomas were less than 2cm in size (87.3%), not associated with metastasis or recurrence (65.5%), and of luminal A and B subtypes (38.2 and 25.5% respectively). Most of *Caspase1*-negative/*EGFR*-overexpression carcinomas were larger than 2cm in size (54.5%), associated with metastasis/recurrence (59.1%), and of TNBLBC subtype (59.1%).

Survival Analysis

Table 3 shows the overall survival (OS) and disease-free survival (DFS) analysis for *Caspase1*, *EGFR*, and combined *Caspase1*/*EGFR* expression patterns using the Log-rank test. Patients with *Caspase1*-positive breast carcinomas had a significantly longer OS compared to patients with *Caspase1*-negative carcinomas (p=0.024). Patients with *EGFR* overexpression breast carcinomas had significantly shorter OS (p=0.001) as compared to patients with *EGFR* low-expression carcinomas. There was a highly significant statistical difference between combined expression groups (p≤0.001), as *Caspase1*-positive/*EGFR*-low-expression pattern was associated with longest OS (64.7months), while Caspase-negative/*EGFR* overexpression pattern was associated with the shortest OS (24.6 months) among the 4 combined expression groups as demonstrated by the Kaplan-Meier curve for OS (Figure 3). There were no significant differences in DFS among different studied *Caspase1* and *EGFR* expression groups.

Discussion

The present study included a cohort of 153 breast carcinoma female patients aiming to detect the immunohistochemical expression of *Caspase1* and *EGFR* in breast carcinoma tissues and to evaluate the association of these markers with the prognostic factors, and with patient's survival. Patients were further classified into combined *Caspase1*/*EGFR* prognostic groups, thus the potential utility of these markers as therapeutic molecular targets in a specific expression group could be identified.

In the current study, 54.2% of breast carcinomas were Caspas1-positive. Compared with the *Caspase1*-negative carcinomas, *Caspase1*-positive carcinomas tended to be of smaller size, less likely associated with metastasis or recurrence, more frequently of luminal A and B subtypes, and less frequently of TNBLBC subtype, and the differences were all statistically significant (p<0.05). Nonetheless, *Caspase1* expression was not related to patient's age, histologic subtype, pathologic grade, stage or Ki67 proliferation index of breast carcinomas. Kaplan-Meier sur¬vival analysis showed that OS time of patients in the group with positive expression of *Caspase1* was significantly higher than that in the group with *Caspase1* negative-expression (p=0.024), though *Caspase1* expression imparted no significant association with the patients DFS. These favorable prognostic associations come to support the data provided by Wu et al. [4] who reported *Caspase1* high expression in 60.1% of breast carcinomas and found this expression to be associated with a smaller tumor size, lower grade and stage and the lower likelihood of nodal metastasis and with a significantly longer total survival time than that in the group with low *Caspase1* expression. This survival advantage was further confirmed in the study by Peng et al. [8] as patients with high *Caspase1* expression had better survival outcomes than those with low expression. In other words, high *Caspase1* serves to suppress breast cancer development and progression, as several studies confirmed a lower expression of *Caspase1* in breast carcinoma tissues compared to normal breast tissues [4, 7, 8, 14].

By the virtue of its activity as a pyrop¬tosis pathway effector protein, *Caspase1* play a crucial role in preventing tumor cells from evading the immune system in the breast carcinoma microenvironment [8]. Moreover, *Caspase1* overexpression induces cellular apoptosis, while its inhibition confers a significantly increased proliferation ability, decreased apoptosis, and increased invasion ability of breast carcinoma cells compared to control groups without *Caspase1* inhibition [7]. It exerts such effects by affecting the cell cycle, immune environment, inflammation, Natural killer (NK) cell regulation of cytotoxicity, p53 expression, the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, the mitogen-activated protein kinase (MAPK) pathway, extracellular matrix, etc., thereby influencing the biological events in breast cancer [8] . Accumulating evidence has demonstrated that *Caspase1* plays a crucial regulatory role in apoptosis and pyroptosis induced by photodynamic therapy in breast cancer cells [5, 14]. Thus, the immunohistochemical detection of this marker in breast carcinoma tissues may play a role in the selection and effectiveness of targeted medicines in breast carcinoma patients.

On the contrary, the expression of *EGFR* in human breast carcinoma tissue is known to be higher than that in the normal breast tissue [5]. It is variously overexpressed in breast cancer, especially in TNBC, with a frequency ranging from 2.7 to 78%, depending

on the primary antibodies used and the molecular subtypes of breast carcinomas included in each study, and both population and genetic differences [10, 13, 20]. In the same context, 32.7% of all breast carcinomas overexpressed membranous *EGFR* in this study. These *EGFR*-overexpressing carcinomas were significantly larger in size and were more frequently TNBLBCs (40%) when compared to the *EGFR* low-expression carcinomas (p=0.004 and 0.018 respectively). Moreover, patients with *EGFR* overexpression breast carcinomas had significantly shorter OS (p=0.001) as compared to patients with *EGFR* low-expression carcinomas, however there was no difference in DFS or the other investigated prognostic variables. Previous studies have similarly shown that *EGFR* expression predominate in TNBC being inversely associated with the ER status [21]. Additionally, *EGFR* expression was significantly associated with poor OS but not with DFS [22], and generally with poor clinical outcomes including recurrence and metastasis and OS in breast cancer patients [23].

Accordingly, several approaches have been developed to target *EGFR* in cancer cells including anti-*EGFR*s antibodies/inhibitors. To date, *EGFR* targeting has not achieved satisfactory clinical results in breast cancer, therefore, clarifying the underlying mechanisms related to the ineffectiveness of *EGFR* inhibitors in breast cancer and developing new *EGFR*-targeted strategies (e.g., combination therapy) is required [24]. Numerous factors, such as drug resistance and lack of proper patient selection may have contributed to the failure of these trials [2, 3, 25].

In this perspective, combined expression of *Caspase1* and *EGFR* may provide better selection of patients who benefit from combination therapies of *EGFR*-inhibitors (such as cetuximab) with an miR-155-5p down-regulators (as antagomir) or with photodynamic therapy via induction of apoptosis and pyroptosis in *EGFR* overexpression carcinomas through *Caspase1*signaling pathway [2, 5]. In this study, 14.4% of breast carcinomas exhibited a *Caspase1*-negative/*EGFR*-overexpression pattern. These carcinomas had the worst prognostic associations being more likely of larger size, associated with metastasis/recurrence and of TNBLBC subtype. Moreover, patients in this group had a shorter OS when compared to other expression patterns of *Caspase1*/*EGFR* and the differences were statistically significant (p=0.002, 0.002, 0.004 and ≤0.001 respectively). Thus, depending on these classification patterns, this group of patients could be the most suitable candidates for the aforementioned combination therapies.

In conclusion, combined *Caspase1*/*EGFR* IHC expression may provide a tool for selection of patients who benefit from combined *EGFR*-inhibitors with miR-155-5p down-regulators or photodynamic therapy via induction of apoptosis/pyroptosis in *EGFR*-overexpression carcinomas through enhanced *Caspase1* signaling.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

This study was not approved by any scientific Body and is not a part of an approved student thesis.

Ethical approval

Ethical approval was obtained from the committed IRB at Mansoura University (Code Number: R.23.09.2337).

Data Availability statement

The data that support the findings of this study are available from the corresponding author on request.

Conflicts of interest

The authors declare that there are no relevant financial affiliations or conflicts of interest to disclose.

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