Original Paper

Assessment of CIP2A and ROCK-1 expression and their prognostic value in breast cancer

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Breast cancer is the most leading cause of cancer death in females worldwide. Identification of novel biomarkers for prognosis is required. Imunohistochemical evaluation of CIP2A and ROCK-1 expressions in 126 breast tissue specimens stratified as 21 ductal hyperplasias, 17 duct carcinoma *in situ* (DCIS) and 88 invasive carcinomas (56 invasive ductal carcinomas NST, 32 invasive lobular carcinomas) was studied. High CIP2A expression was detected in 48.9% of invasive carcinomas. CIP2A overexpression was significantly related to Nottingham prognostic index (NPI) ($p = 0.011$), stage ($p = 0.01$), ER negativity ($p = 0.031$), PR negativity ($p = 0.048$), and HER-2 positivity ($p = 0.02$). CIP2A was significantly overexpressed in triple-negative breast cancer (TNBC) ($p = 0.004$). ROCK-1 expression was detected in 54.5% of invasive carcinomas. Statistically significant associations were observed between ROCK-1 expression and NPI ($p = 0.032$), stage ($p = 0.002$), ER negativity ($p = 0.012$), PR negativity ($p = 0.023$), HER-2 positivity ($p = 0.016$), and TNBC subtype ($p = 0.033$). A positive association between CIP2A and ROCK-1 expressions ($p < 0.0001$) was documented. There was a significant association between shorter overall survival and high CIP2A and positive ROCK-1 expressions ($p < 0.0001$) and ($p < 0.0001$). CIP2A and ROCK-1 expressions could be used as markers for the poor prognosis of breast cancer.

Key words: breast cancer, CIP2A, ROCK-1, immunohistochemistry, prognosis.

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 11.6% of the total cancer cases and 6.6% of the total cancer deaths in 2018 [1].

In Egypt, breast cancer is the most common malignant tumor among women, constituting 32.04% of total malignancies. The frequency of breast cancer observed in different regions of Egypt was about 33.8%, 26.8%, and 38.7% of cancer cases in upper, middle, and Lower Egypt zones, respectively [2].

Breast cancer includes several biological subtypes with a wide spectrum of clinical, pathologic and molecular features resulting in different prognostic and therapeutic implications [3]. Despite the advancement in molecular studies, the prognosis and therapeutic targets of breast cancer are still depending on traditional markers such as tumor stage and hormonal receptor status [4, 5]. Thus, identification of novel biomarkers for prognosis, prediction, and therapeutic purposes is essentially required [6].

Triple-negative breast cancer (TNBC) is a subtype of breast cancer in which the estrogen receptor together with progesterone receptor are not expressed, and human epidermal growth factor receptor 2 is not amplified or overexpressed [7]. Generally, TNBCs are more aggressive tumors with a high metastatic potential and a shorter time

of recurrence [8]. Lacking responses for both hormonal and immunotherapeutic treatment modalities makes TNBC in a great demand for new therapeutic targets [7].

Protein phosphatase 2A (PP2A) accounts for most of the serine/threonine phosphatase activity in the cell and it acts as a tumor suppressor by inhibiting the activity of several oncogenic signaling pathways [9, 10]. Cancerous Inhibitor of PP2A (CIP2A) binds to PP2A and inhibits its phosphatase functions resulting in the tumorogenic transformation of cells [11]. CIP2A stabilizes c-Myc by inhibiting PP2A-mediated dephosphorylation of Myc at serine 62 [12]. The expression o fc-Myc rapidly induces the activation of cyclin E-cdk2 kinase activity, with the simultaneous release of p27Kip1 from cdk2 complexes, which is essential for G1/S transition and cell cycle progression [12].

CIP2A is overexpressed at a high frequency in a number of tumors and expression levels are independent markers for long-term outcomes in many of these tumors [11]. The clinical relevance of CIP2A overexpression as a prognostic marker has been established in various solid and hematological malignancies, including, chronic myelogenous leukemia, bladder carcinoma, non-small cell lung cancer, multiple myeloma, and endometrioid adenocarcinoma [13, 14, 15, 16, 17]. Regarding breast cancer, CIP2A expression has been correlated with aggressiveness and also predicts a worse prognosis [18].

Alteration in the actin cytoskeleton can cause modifications in cell adhesion, contraction, migration, and invasion. Additionally, it can affect gene expression, the cell cycle, and remodeling of the extracellular matrix (ECM) [19, 20]. ROCK (Rho-associated coiled-coil kinase) belongs to the AGC family of serine/threonine protein kinases and it consists of two isoforms, ROCK-1 and ROCK-2 [21, 22]. ROCK family is mainly activated through interaction with the small Rho GTPases, including RhoA, RhoB, and RhoC [23].

The main role of the ROCK family is increasing the stabilization of actin filaments and the generation of actin-myosin contractility through phosphorylation of multiple downstream substrates. The generated contractile force influences cell behaviors including; contraction, motility, survival, and proliferation [24]. Elevated ROCK-1 and/or ROCK-2 expressions have been observed in several human cancers, in which they are associated with poor prognosis [25, 26, 27, 28]. It has also been found that there are some beneficial roles of ROCK inhibition on tumor volume, invasiveness, and metastatic potential [29, 30].

Concerning breast cancer, high expression of ROCK-1 has been found to be significantly correlated with poor prognostic indicators including high grade, advanced stage, positive nodal metastasis, and shortened survival [31, 32, 33].

To our knowledge, no previous studies have focused on the association between CIP2A and ROCK-1 in breast cancer. The aim of this work is to investigate the immunoexpression of CIP2A, and ROCK-1 in breast cancer and to correlate their expressions with different clinicopathological characteristics in order to elucidate their prognostic value and possible therapeutic implications in patients with breast cancer.

Material and methods

Patients and tissue specimens

This study included 126 formalin-fixed, paraffin-embedded tissue specimens distributed as 21 ductal hyperplasia cases, 17 duct carcinoma *in situ* (DCIS) cases and 88 invasive carcinomas (56 invasive ductal carcinomas NST, 32 invasive lobular carcinomas). They were selected from the archives of the Department of Pathology, Minia University Hospital and Minia Oncology Center between November 2013 and November 2018 according to the availability of paraffin blocks and full clinicopathological data. This study was conducted in accordance with the 1975 Declaration of Helsinki. The histopathological categorization of tissue samples is illustrated in (Table I).

The clinicopathologic characteristics of patients of invasive breast cancers were extracted from the medical records including patients age at diagnosis, menopausal status, lymph node positivity, TNM stage, histological type, estrogen receptor (ER) status, progesterone receptor (PR) status and Her2/neu (human epidermal growth factor receptor 2). Paraffin blocks of the specimens were subjected to hematoxylin and eosin staining (HE), for reviewing the diagnosis. Overall survival was calculated in months from the date of diagnosis and ended with the time of the tumor-related death or with the last follow-up visit of the patient.

Immunohistochemical procedures

Paraffin-embedded tissue sections of 5 μm thickness were deparaffinized in xylene and rehydrated through descending grades of alcohol. For antigen retrieval, the slides were heated in 10 mmol/l sodium citrate buffer (pH 6.0) using a microwave oven for 20 minutes at 750 W. Blocking of endogenous peroxidase activity was done by incubation in 0.3% hydrogen peroxide in methanol for 30 minutes.

The slides were then incubated overnight at 4°C in the humidity chamber with CIP2A mouse monoclonal primary antibody (clone2G10-3B5:

Table I. The histopathological categories of study samples

Tissue samples (n = 126)	n (%)
Invasive breast cancer	
IDC, NST	56 (44.4%)
ILC	32 (25.4%)
DCIS	
Low and intermediate-grades	7 (5.6%)
High-Grade	10 (7.9%)
Benign lesions	
Usual ductal hyperplasia	14 (11.1%)
Atypical ductal hyperplasia	7 (5.6%)

sc-80659, 1:50 dilution, Santa Cruz Biotechnology Corporation, USA). Incubation with ROCK-1 mouse monoclonal primary antibody was done at 4°C overnight in the humidity chamber (clone G-6: SC-17794, 1:50 dilution, Santa Cruz Biotechnology Corporation, USA). Then, secondary biotin-conjugated antibody and the enzyme conjugate reagent was applied for 30 minutes each (Lab Vision Corporation, CA, USA).

The slides were stained with Diaminobenzidine tetrachloride (DAB) plus substrate-chromogen solution (Lab Vision Corporation, CA, USA), and then counterstained in hematoxylin. The positive control for CIP2A was human kidney tissue, while normal liver tissue was used as a positive control for ROCK-1. The replacement of the primary antibody by PBS solution was served as a negative control.

Immunohistochemical assessment

Assessing the expression of both markers was performed by three independent pathologists. Regarding CIP2A, the intensity of staining was categorized as follows; 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The percentage of positive cells was categorized as follows: 1 (in 1-25% of cells), 2 (in 26-50% of cells), 3 (in 51-75% of cells), or 4 (in 76-100% of cells). The final combined score (0-12) was obtained by multiplying both the percentage and intensity scores of each tissue section. Sections with combined scores ≤ 3 were considered as low CIP2A expression while those having combined scores > 3 were considered as a high expression [15, 34]. For the ROCK-1 expression assessment, the staining intensity was scored as follows: 0 (no staining), 1 (brown) and 2 (dark brown). While for a percentage of positive cells: 0 for positive staining cells < 5%, 1 for 5-25%, 2 for 26-50% and 3 for above 50% cells with positive staining. The final combined score of ≥ 2 corresponds to positive, while score < 2 corresponds to negative staining [28, 35].

Statistical analysis

Data were checked, coded, entered, and analyzed by using SPSS (The Statistical Package for Social Sciences) version 17.0 software. Descriptive methods used are mean, standard deviation (SD), frequency distribution, and cross-tabulation. Significance tests: χ^2 test was used for categorical data. Moreover, Spearman's rho correlation was performed to detect the correlation between the two markers. Statistical significance was set at p-value ≤ 0.05.

Survival analysis

Kaplan-Meier survival curves and log-rank test statistics were employed to evaluate overall patients' survival and their differences. Multivariate regression analysis was carried out using Cox regression to assess the specific impact of each variable on survival in the presence of other variables. Only variables of significant value from the univariate analysis were entered into the Cox regression analysis.

Results

Patient characteristics

Patients' age range was from 23 to 79 years, with a mean age (51.1 ±9.8). Based on the ER, PR and HER2 IHC expression profile, breast cancers were stratified into 4 molecular subtypes. Luminal A: ER+ and/or PR+, HER2−; Luminal B: ER+ and/or PR+, HER2+; HER2-overexpressing: ER− and PR−, HER2+; Basal-like/TNBC: ER−, PR−, HER2−. Histological grading and Nottingham prognostic index (NPI) were applied for IDC cases.

Immunohistochemical expression pattern of CIP2A

The expression of CIP2A was observed in the cytoplasm of all positive cases. Normal breast tissue and all benign lesions didn't exhibit any CIP2A reactivity. Meanwhile, high CIP2A expression was detected in 3 out of 17 cases (17.6%) of DCIS and in 43 out of 88 cases (48.9%) of invasive breast cancers (Fig. 1). The difference between CIP2A expression in invasive lesions compared to DCIS areas was statistically significant (p = 0.035; Table II).

Correlation between CIP2A expression and different clinicopathologic data

As shown in Table III, no association was found between CIP2A expression and patients' age, histologic subtype, menopausal status, laterality, or parity.

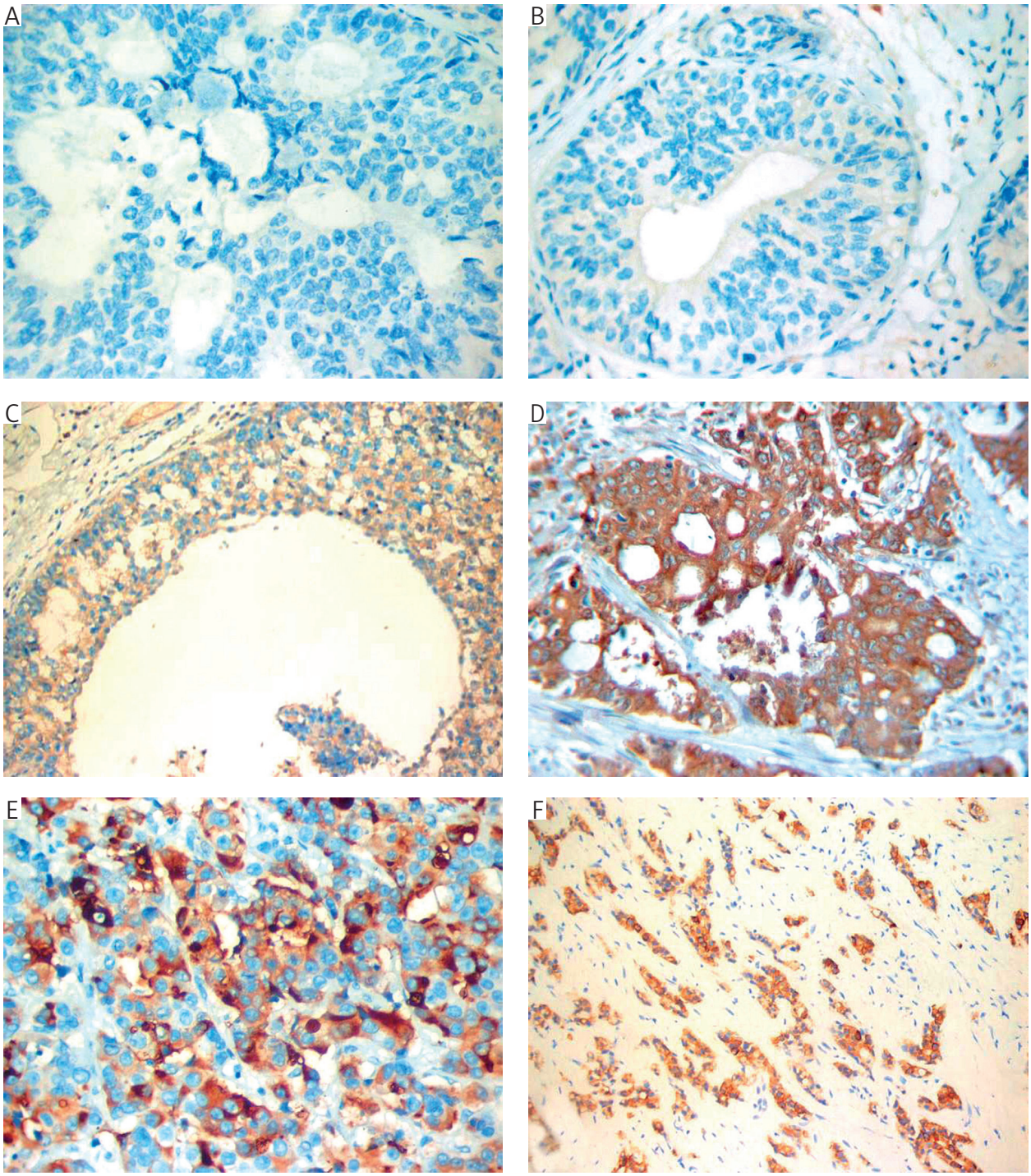


Fig. 1. Immunohistochemical expression of CIP2A in different breast lesions: A) negative CIP2A expression in atypical ductal hyperplasia; B) negative CIP2A expression in duct carcinoma *in situ*; C) weak positive expression of CIP2A in duct carcinoma *in situ*; D) strong positive expression of CIP2A in grade III invasive ductal carcinoma, NST; E) moderate positive expression of CIP2A in grade II invasive ductal carcinoma; F) moderate positive expression of CIP2A in invasive lobular carcinoma. Original magnification 400× in figures A, B, E; 200× in figures C, D, F (DAB was used as the chromogen and haematoxylin as counterstain)

CIP2A overexpression was significantly related to high histological grade ($p = 0.011$), NPI ($p = 0.011$), tumor size ($p = 0.038$), positive lymph node metastasis ($p = 0.016$), and more advanced stage ($p = 0.01$). Furthermore, significant association was observed between high CIP2A expression and, ER negativity ($p = 0.031$), PR negativity ($p = 0.048$), and HER-2 positivity ($p = 0.02$). Also, CIP2A was significantly over expressed in TNBC compared to other molecular subtypes ($p = 0.004$).

Table II. CIP2A expression in invasive breast cancer and DCIS

Cases	n = 105	CIP2A expression		p-value
		Low (n = 59)	High (n = 46)	
	n (%)	n (%)	n (%)	
Invasive cancers	88 (83.8%)	45 (51.1%)	43 (48.9%)	
DCIS	17 (16.2%)	14 (82.3%)	3 (17.6%)	0.035*

*DCIS: duct carcinoma in situ. Test of significance: χ^2 test. P-value ≤ 0.05 is considered significant**

Table III. The relationship between CIP2A expression and clinicopathological characteristics of malignant invasive lesions

Clinicopathological characteristics	n = 88	CIP2A expression		p-value
		Low (n = 45)	High (n = 43)	
	n (%)	n (%)	n (%)	
Mean Age ±SD				
< 51.1 ±9.8	52 (59%)	29 (64.4%)	23 (53.5%)	**0.41**
≥ 51.1 ±9.8	36 (41%)	16 (35.6%)	20 (46.5%)	
Histopathological Types				
IDC, NST	56 (63.6%)	27 (60%)	29 (67.4%)	**0.61**
ILC	32 (36.4%)	18 (40%)	14 (32.6%)	
Menopausal Status				
Premenopause	50 (56.8%)	30 (66.7%)	20 (46.5%)	**0.09**
Postmenopause	38 (43.2%)	15 (33.3%)	23 (53.5%)	
Laterality				
Right	29 (33%)	16 (35.6%)	13 (20.2%)	
Leftt	46 (52.3%)	24 (53.3%)	22 (51.2%)	
Bilateral	13 (14.7%)	5 (11.1%)	8 (18.6%)	**0.6**
Parity				
Multipara	74 (84.1%)	39 (86.7%)	35 (81.4%)	**0.7**
Nulipara	14 (15.9%)	6 (13.3%)	8 (18.6%)	
Grade (for IDC, NST)	(n = 56)	(n = 27)	(n = 29)	
Grade 1	6 (18.7%)	5 (18.5%)	1 (3.4%)	
Grade 2	26 (48.4%)	16 (59.3%)	10 (34.5%)	
Grade 3	24 (42.9%)	6 (22.2%)	18 (62.1%)	**0.011***
NPI (for IDC, NST)	(n = 56)	(n = 27)	(n = 29)	
Good	8 (14.3%)	6 (22.2%)	2 (6.9%)	
Moderate	22 (39.3%)	14 (51.9%)	8 (27.6%)	
Poor	26 (48.4%)	7 (25.9%)	19 (65.5%)	**0.011***
Pathological Size				
pT1	18 (20.5%)	13 (28.9%)	5 (11.6%)	
pT2	30 (34%)	17 (37.8%)	13 (30.2%)	
pT3	40 (45.5%)	15 (33.3%)	25 (58.2%)	**0.038***
Lymph Node Status				
N0	37 (42%)	25 (55.6%)	12 (27.9%)	
N1-3	51 (58%)	20 (44.4%)	31 (72.1%)	**0.016***

Table III. Cont.

Clinicopathological characteristics	n = 88	CIP2A expression		p-value
		Low (n = 45)	High (n = 43)	
	n (%)	n (%)	n (%)	
Stage				
Stage I	12 (13.6%)	9 (20%)	3 (7%)	
Stage II	53 (60.3%)	30 (66.7%)	23 (53.5%)	
Stage III	23 (26.1%)	6 (13.3%)	17 (39.5%)	0.01*
Estrogen receptors status				
ER+	40 (45.5%)	26 (57.8%)	14 (32.6%)	
ER–	48 (54.5%)	19 (42.2%)	29 (67.4%)	0.031*
Progesterone receptors status				
PR+	37 (42%)	24 (53.3%)	13 (30.2%)	
PR–	51 (58%)	21 (46.7%)	30 (69.8%)	0.048*
Her-2 receptors status				
Her-2+	41 (46.6%)	15 (33.3%)	26 (60.4%)	
Her-2–	47 (53.4%)	30 (66.7%)	17 (39.6%)	0.02*
Molecular subtype				
Luminal A	23 (26.1%)	15 (33.3%)	8 (18.6%)	
Luminal B	21 (23.9%)	15 (33.3%)	6 (14%)	
Her 2+	18 (20.5%)	9 (20%)	9 (20.9%)	
Triple-negative subtype	26 (29.5%)	6 (13.3%)	20 (46.5%)	0.004**
Triple-negative subtype				
Yes	26 (29.5%)	6 (13.3%)	20 (46.5%)	
No	62 (60.5%)	39 (86.7%)	23 (53.5%)	**0.002****

*NPI – Nottingham prognostic index. Test of significance: χ^2 test. P-value ≤ 0.05 is considered significant**

Table IV. ROCK-1 expression in invasive breast cancer and DCIS

Cases	n = 105	ROCK-1 expression		p-value
		Negative (n = 55)	Positive (n = 50)	
	n (%)	n (%)	n (%)	
Invasive cancers	88 (83.8%)	40 (45.5%)	48 (54.5%)	
DCIS	17 (16.2%)	15 (88.2%)	2 (11.8%)	0.003*

*DCIS – duct carcinoma in situ. Test of significance: χ^2 test. P-value ≤ 0.05 is considered significant**

Immunohistochemical expression pattern of ROCK-1

ROCK-1 expression was detected in the cytoplasm. ROCK-1 expression was not detected in normal breast tissue, while 2 out of 21 cases (9.5%) of benign lesions displayed ROCK-1 expression. Two out of 17 cases (11.8%) of DCIS and 48 out of 88 cases (54.5%) of invasive carcinoma were positive for ROCK-1 (Fig. 2). ROCK-1 was more obviously expressed in invasive breast cancer compared to DCIS cases (p = 0.003; Table IV).

Correlation between ROCK-1 expression and different clinicopathologic data

As shown in Table V, ROCK-1 expression was not associated with patient age, histologic subtypes, menopausal status, laterality, parity, or tumor size. Significant association was observed between ROCK-1 expression and high histological grade (p = 0.011), NPI (p = 0.032), positive lymph node metastasis (p = 0.007), and more advanced stage (p = 0.002). In addition, significant association was observed between ROCK-1

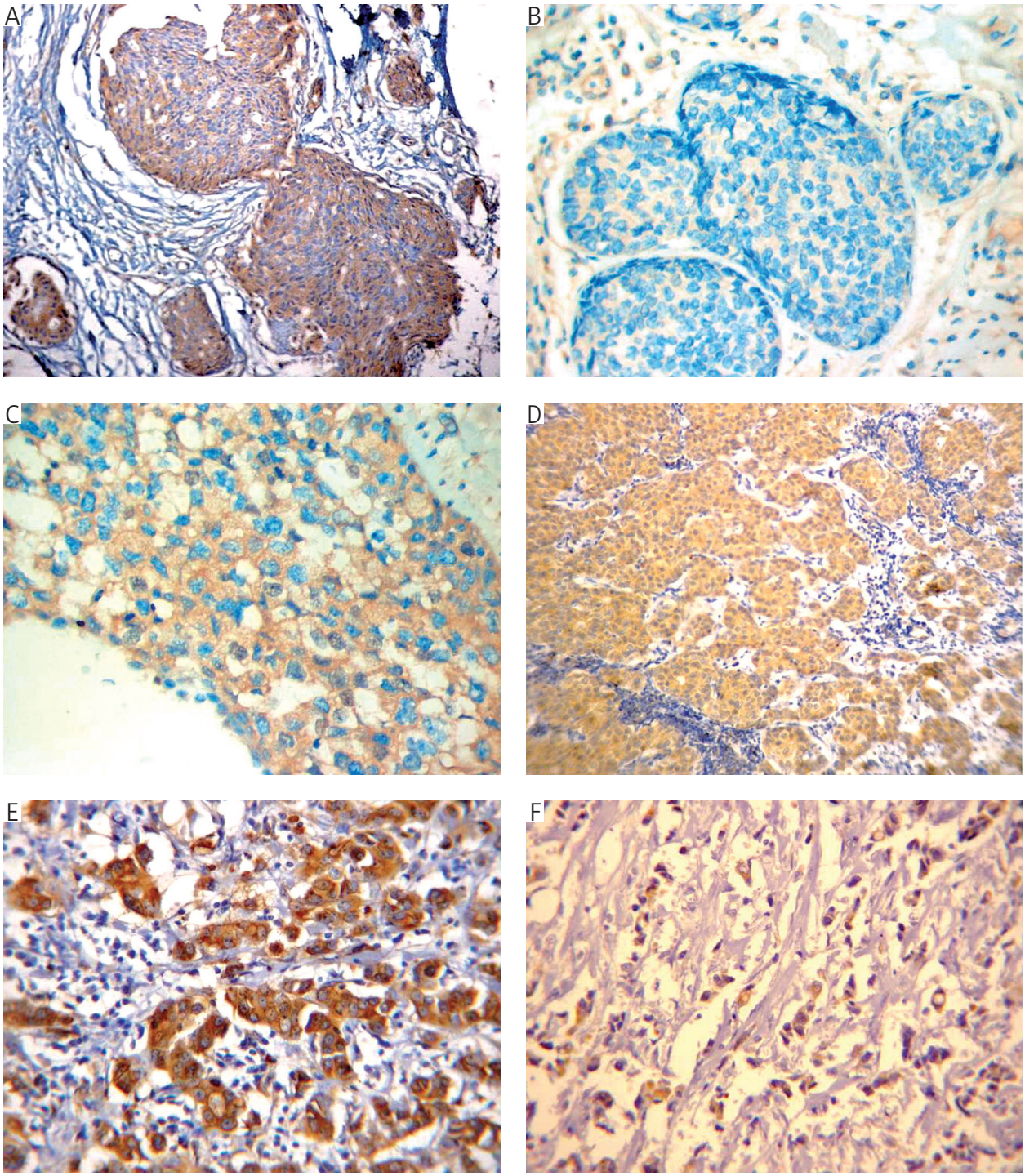


Fig. 2. Immunohistochemical expression of ROCK-1 in different breast lesions: A) positive ROCK-1 expression in atypical ductal hyperplasia; B) negative ROCK-1 expression in duct carcinoma *in situ*; C) positive (brown) expression of ROCK-1 in duct carcinoma *in situ*; D) positive (brown) expression of ROCK-1 in grade II invasive ductal carcinoma; E) positive (dark brown) expression of ROCK-1 in grade II invasive ductal carcinoma, NST; F) positive (brown) expression of ROCK-1 in invasive lobular carcinoma. Original magnification 400× in figures A, B, E; 200× in figures C, E, F (DAB was used as the chromogen and haematoxylin as counterstain)

Table V. The relationship between ROCK-1 expression and clinicopathological characteristics of malignant invasive lesions (n = 88)

Clinicopathological characteristics	n = 88	ROCK-1 expression		p-value
		Negative (n = 41)	Positive (n = 47)	
	n (%)	n (%)	n (%)	
Mean age ±SD				
< 51.1 ±9.8	52 (59%)	28 (68.3%)	24 (51.1%)	
≥ 51.1 ±9.8	36 (41%)	13 (31.7%)	23 (48.9%)	**0.15**
Histopathological types				
IDC	56 (63.6%)	26 (63.4%)	30 (63.8%)	**0.97**
ILC	32 (36.4%)	15 (36.6%)	17 (36.2%)	
Menopausal status				
Premenopause	50 (56.8%)	28 (68.3%)	22 (46.8%)	**0.07**
Postmenopause	38 (43.2%)	13 (31.7%)	25 (53.2%)	
Laterality				
Right	29 (33%)	11 (36.7%)	18 (38.3%)	
Leftt	46 (52.3%)	25 (61%)	21 (44.7%)	**0.31**
Bilateral	13 (14.7%)	5 (12.3%)	8 (17%)	
Parity				
Multipara	74 (84.1%)	34 (82.9%)	39 (85.1%)	**0.78**
Nulipara	14 (15.9%)	7 (17.1%)	7 (14.9%)	
Grade (only for IDC, NST)	(n = 56)	(n = 26)	(n = 30)	
Grade 1	6 (18.7%)	5 (19.2%)	1 (3.3%)	
Grade 2	26 (48.4%)	15 (57.7%)	11 (36.7%)	**0.011***
Grade 3	24 (42.9%)	6 (23.1%)	18 (60%)	
NPI (only for IDC, NST)	(n = 56)	(n = 26)	(n = 30)	
Good	8 (14.3%)	7 (26.9%)	1 (3.3%)	
Moderate	22 (39.3%)	10 (38.5%)	12 (40%)	**0.032***
Poor	26 (48.4%)	9 (34.6%)	17 (56.7%)	
Pathological size				
pT1	18 (20.5%)	10 (24.3%)	8 (17%)	
pT2	30 (34%)	14 (34.1%)	16 (33.1%)	**0.65**
pT3	40 (45.5%)	17 (41.6%)	23 (48.9%)	
Lymph node status				
N0	37 (42%)	24 (58.4%)	13 (27.7%)	**0.007****
N1-3	51 (58%)	17 (41.6%)	34 (72.3%)	
Stage				
Stage I	12 (13.6%)	10 (24.3%)	2 (4.3%)	
Stage II	53 (60.3%)	26 (63.4%)	27 (57.4%)	**0.002***
Stage III	23 (26.1%)	5 (12.3%)	18 (38.3%)	
Estrogen receptors status				
ER+	40 (45.5%)	25 (61%)	15 (31.9%)	**0.012***
ER–	48 (54.5%)	16 (39%)	32 (68.1%)	

Table V. Cont.

Clinicopathological characteristics	n = 88	ROCK-1 expression		p-value
		Negative (n = 41)	Positive (n = 47)	
	n (%)	n (%)	n (%)	
Progesterone receptors status				
PR+	37 (42%)	23 (56.1%)	14 (29.8%)	**0.023***
PR–	51 (58%)	18 (43.9%)	33 (70.1%)	
Her-2 receptors status				
Her-2+	41 (46.6%)	13 (31.7%)	28 (59.6%)	**0.016***
Her-2–	47 (53.4%)	28 (68.3%)	19 (40.4%)	
Molecular subtype				
Luminal A	23 (26.1%)	13 (31.7%)	10 (21.3%)	
Luminal B	21 (23.9%)	13 (31.7%)	8 (17%)	**0.033***
Her-2+	18 (20.5%)	9 (22%)	9 (19.1%)	
Triple-negative subtype	26 (29.5%)	6 (14.6%)	20 (42.6%)	
Triple-negative subtype				
Yes	26 (29.5%)	7 (17.1%)	19 (40.4%)	**0.031***
No	62 (60.5%)	34 (82.9%)	28 (59.6%)	

*NPI – Nottingham prognostic index. Test of significance: χ^2 test. P-value ≤ 0.05 is considered significant**

Table VI. Multivariate analyses for overall survival in 88 patients with malignant invasive lesions

Variables	B	SE	p-value	HR (95% CI)
Histopathological type	0.771	0.262	0.003	2.162 (1.292-3.616)
Grade	0.507	0.319	2.535	1.661 (0.889-1.752)
NPI (only for IDC)	0.169	0.200	0.399	1.184 (0.800-1.752)
Pathological size	0.078	0.196	0.689	1.081 (0.737-1.586)
Stage	0.275	0.195	0.159	1.317 (0.898-1.930)
Estrogen receptor status	–0.383	0.394	0.330	0.682 (0.315-1.475)
Progesterone receptor status	–0.044	0.367	0.904	0.957 (0.466-1.963)
Triple-negative subtype	0.157	0.320	0.623	0.706 (0.152-0.604)
CIP2A expression	–0.349	0.345	0.312	0.706 (0.359-1.388)
ROCK-1 expression	–1.194	0.352	0.001	0.303 (0.152-0.604)

B – regression coefficient; SE – standard error; HR – hazard ratio; CI – confidence interval; NPI –Nottingham prognostic index; p-value ≤ 0.05 is considered significant. Cox regression test is used

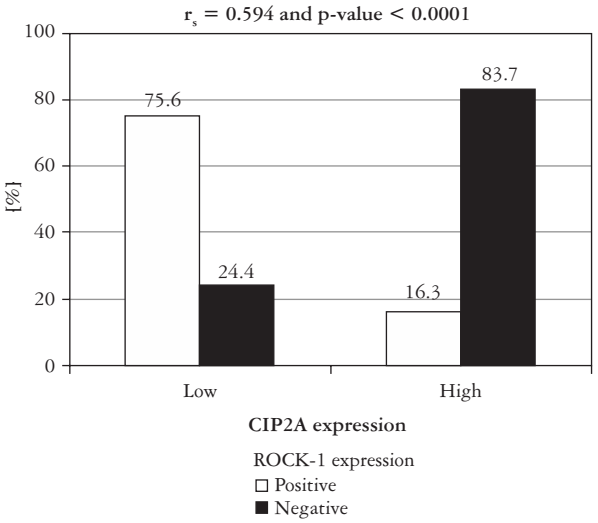
expression and, ER negativity (p = 0.012), PR negativity (p = 0.023), HER-2 positivity (p = 0.016), and TNBC subtype (p = 0.033).

Association between CIP2A and ROCK-1 expressions

We further evaluated the association between CIP2A and ROCK-1 expressions in invasive breast carcinoma cases. Our results showed positive association between CIP2A and ROCK-1 expressions (r_s = 0.594; p < 0.0001) (Fig. 3).

Correlation between CIP2A and ROCK-1 expressions and survival of patients

Thirty two patients (18.25% of all cases studied; 36.36% of invasive cancer cases) died during 5-year follow-up. The median overall survival period was 41.5 months (range 14-60 months). Using Kaplan- Meier method and log-rank test, there was a significant association between shorter overall survival of breast cancer and larger tumor size (p = 0.005), tumor subtype (p = 0.005), histologic grade (p = 0.030), high stage (p = 0.027),



Test of significance: Spearman's rho correlation. P-value < 0.05 is considered significant

Figure 3. Relation between CIP2A and ROCK-1 expression in invasive breast carcinoma cases (n = 88)

NPI (p = 0.003), estrogen receptor negativity (p < 0.0001), progesterone receptor negativity (p = 0.029), triple-negative subtype (p = 0.048), high CIP2A expression (p < 0.0001) and high ROCK-1 expression (p < 0.0001). Postoperative mortality was observed in 18/56 patients with invasive ductal carcinoma, NST (32.14%) and in 14/32 patients with invasive lobular carcinoma (43.75%) during 5-year follow-up. On the other hand, no significant association was found between overall survival of breast cancer cases and both age (p = 0.733) and HER-2 expression (p = 0.723).

In multivariate analysis, only ductal tumor subtype and ROCK-1 expression were independent predictor factors for reduced overall survival (p = 0.0003; 0.001 respectively; as shown in Table VI).

Discussion

The present study has analyzed the expression patterns of CIP2A and ROCK-1 in different breast lesions. High cytoplasmic expression of CIP2A was detected in 43/88 (48.9%) invasive carcinoma cases. In DCIS, 3/17 (17.6%) cases displayed high CIP2A expression. We have reported significant CIP2A overexpression in both invasive breast carcinoma and DCIS cases when compared to benign lesions. Furthermore, adjacent normal breast tissues did not display any CIP2A expression. This was in line with another study that reported high cytoplasmic expression of CIP2A protein in 39% of 33 breast carcinomas without expression in normal mammary tissue [36]. Therefore, the previous findings can suggest a possible association of CIP2A overexpression with cancer progression and aggressiveness.

In the present study, CIP2A overexpression was significantly related to high histological grade, NPI, positive lymph node metastasis, advanced stage, and shorter overall survival. Furthermore, a significant association was observed between high CIP2A expression and HER-2 positivity, ER and PR negativity. Many studies reported association of high CIP2A expression with poor prognostic factors [36, 37, 38, 39] and recurrence after treatment [18].

Concerning molecular subtypes, CIP2A was significantly overexpressed in TNBC. This finding was in agreement with a previous study that demonstrated overexpression of CIP2A mRNA and protein in the TNBC cell line and tissue compared with receptor-positive cell lines and cells [39]. Moreover, CIP2A depletion in TNBC cell lines resulted in inhibition of proliferation and invasion, on one hand, and induction of apoptosis and autophagy on the other hand. Another study showed that increased CIP2A expression was significantly related to basal-like and HER2+ breast cancers [40].

Our results displayed more positive expression of ROCK-1 in invasive breast cancer than both DCIS and benign lesions to statistically significant levels (p = 0.003). In accordance with our results, previous studies showed no ROCK-1 expression in normal breast tissues, while high ROCK-1 expression was detected especially in patients with metastasis [33, 41, 42]. They also concluded that ROCK-1inhibition can decrease cell migration, proliferation, and metastasis [43, 44]. Taken together, the previous findings can confirm the obvious role of ROCK-1 in promoting breast cancer metastasis.

We found significant associations between ROCK-1 expression and higher histological grade, NPI, positive lymph node metastasis, advanced stage, and reduced overall survival. Furthermore, we demonstrated significant associations between ROCK-1 expression and ER negativity, PR negativity, HER-2 positivity and also with TNBC. Many publications have demonstrated significant association of ROCK-1 expression with disadvantageous prognostic parameters in breast cancer [33, 41, 42, 45].

In our study, the association between CIP2A and ROCK-1 expressions in breast cancer was studied and the positive association between these proteins could suggest possible link between both CIP2A and ROCK-1 in progression of breast cancer. This finding needs future research work to confirm it.

The possible explanation for such positive association between CIP2A and ROCK-1 overexpression in breast cancer could be postulated based on their roles in cell cycle progression. On one hand, ROCK signaling is implicated in cell cycle progression through regulation of several cell cycle regulatory proteins by

different mechanisms. One of these mechanisms is through reduction of p27 levels, which in turn stimulates G1/S cell cycle progression [46]. Moreover, Rho activity was found to phosphorylate c-Myc and promote its stability and transcriptional activity in breast and prostate cancers [43, 47]. On the other hand, it has previously been shown that CIP2A inhibits c-Myc dephosphorylation and stabilizes its activity, resulting in release of p27 from cyclin E-cdk2 kinase complexes, which is needed for G1/S transition and cell cycle progression [12]. Also, CIP2A knockdown resulted in increased p27 level and thus resulted in arrest of cell cycle in TNBC [39].

Regarding the role of targeting CIP2A towards the treatment of breast cancer, the results of previous studies confirmed that Arctigenin inhibits triple-negative breast cancers proliferation, progression and invasion by targeting CIP2A to reactivate protein phosphatase 2A [48]. Moreover, CIP2A mediate bortezomib-induced apoptosis in TNBC cells. Thus, CIP2A may be a potential therapeutic target in TNBC [49].

Previous studies reported that ROCK1 expression was decreased by treatment with EGFR inhibitor, MEK inhibitor and Integrin β1 function blocking antibody. Two ROCK inhibitors (Y-27632 and Fasudil, 30 μM) caused a decrease in both EGFR and Integrin β1 protein levels. Furthermore, the ROCK inhibitor Y-26735 reduced the levels of GLUT3 and LDHA proteins in breast cancer cells. These observations suggest that the ROCK signaling pathways are integrated with other signaling pathways. Disruption of these pathways leads to a malignant phenotype observed in breast cancer cells [50, 51]. Thus ROCK inhibitors may be valuable for prevention of invasion and metastasis [52].

Conclusions

In summary, our research demonstrates that high CIP2A and ROCK-1 expressions are associated with breast cancer progression. Only, ROCK-1 expression was associated with tumor aggressiveness, poor prognosis and decreased overall survival. Our results indicate that CIP2A together with ROCK-1 might be promising predictive biomarkers and can be possible targets for future therapeutic regimens in breast cancer treatment. Further studies are required to understand in depth the underlying molecular and cellular mechanisms of CIP2A and ROCK-1 in breast cancer.

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