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Role of CNK1, Ephrin B1, GPR19 and SMURF1 in breast cancer early diagnosis, metastasis and drug resistance

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Abstract

Background: The extracellular signal-regulated kinase (ERK) pathway is a key signaling pathway involved in the regulation of normal cell proliferation, survival and differentiation. However, aberrant regulations of the ERK pathway contribute to cancer and other human diseases.

Objective: This study was designed to investigate the role of some ERK pathway effectors such as the connector enhancer of kinase suppressor of Ras1 (CNK1), Ephrin B1, G protein-coupled receptor 19 (GPR19) and SMAD ubiquitination regulatory factor 1 (SMURF1) in breast cancer (BC) diagnosis and metastasis risk prediction.

Methods: The study involved 50 ($ER^-PR^-Her2^+$=6, $ER^+PR^+Her2^+$=11, $ER^-PR^-HER2^-$=8, $ER^+PR^+Her2^-$=25) newly diagnosed BC patients, 15 chemotherapy resistant BC patients, 15 benign breast tumor patients and 10 controls. All total 65 BC patients (including the chemotherapy resistant group) were subdivided into two groups: metastatic BC (17 patients), and non-metastatic BC group (48 patients). CNK1, Ephrin B1, GPR19 and SMURF1 serum levels were analyzed using ELISA.

Results: The study revealed significantly higher serum levels of CNK1, Ephrin B1, GPR19 and SMURF1 in all malignant groups ($ER^-PR^-Her2^+$, $ER^+PR^+Her2^+$, $ER^-PR^-HER2^-$, $ER^+PR^+Her2^-$), as well as a significant elevation in the chemotherapy resistant BC group as compared to non-resistant group ($P < 0.001$). They also revealed excellent value for de novo BC diagnosis and metastasis prediction.

Conclusion: CNK1, Ephrin B1, GPR19 and SMURF1 may be considered as novel biomarkers for BC diagnosis and prediction of metastasis risk.

Keywords: Breast cancer; ERK pathway; CNK1, Ephrin B1; GPR19; SMURF1

1. Introduction

Cancer is a main public health challenge whose prevalence and mortality are rapidly increasing globally. It is considered as the primary cause of death and the central obstacle to increasing life expectancy in all the world countries this century [1]. Cancer development and progression can take place by several mechanisms such as maintaining proliferative signaling [2], escaping growth suppressors, apoptosis and autophagy [3-5] , stimulating invasion and metastasis and deregulating cellular energetics and metabolism [6].

Concerning females, breast cancer (BC) is reported as the major frequently diagnosed cancer and the main reason for cancer related death [7, 8]. Approximately around 15% of BC patients suffer an aggressive disease with distant metastases within 3 years from initial diagnosis [9]. The incidence rates are high in both developed and developing countries [10]; however, the mortality rate is relatively more elevated in less developed countries due to late detection, inadequate lifestyles and limited access to treatment facilities [11].

The extracellular signal regulated kinase (ERK) signaling pathway is a key determining factor in the

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control of various cellular activities such as motility, proliferation, differentiation and survival. This pathway is activated by diverse mechanisms and is often upregulated in human tumors [12]. Scaffold proteins control when and where ERK is activated [13].

The connector enhancer of kinase suppressor of Ras 1 (CNK1) is a multidomain scaffold protein, which is necessary for Ras activation of the Raf kinase [14]. Current data submitted that CNK1 has numerous roles in cancer progression. Several reports reveal that it interacts with different tumor suppressors and others designate its scaffolding protein connections as oncogenic. Moreover, CNK1 is an Ephrin B1-associated protein playing together a role in cancer cell proliferation and net cancer cell migration [15].

Dysfunction of G protein-coupled receptors (GPRs) is well-known to play a key role in the development of a diversity of diseases, involving cancer induction and growth. Up-regulated expression of GPR19 leads mesenchymal-like BC cells to implement an epithelial-like phenotype, and changes in functional behavior. Moreover, GPR19 plays a possible role in metastasis by stimulating mesenchymal-epithelial transition through the MAPK/ERK pathway [16].

Activation of the MAPK/ERK pathway is a crucial signal transduction occurrence followed by estrogen-mediated cell propagation. Current studies have demonstrated that constant activation of ERK plays a key role in cell migration and tumor development [17], as well as inducing SMAD ubiquitination regulatory factor 1 (SMURF1) expression, a well-known E3 ubiquitin ligase, which is involved in the ubiquitination and proteasomal degradation of substrate proteins. It is thought to take part in epithelial to mesenchymal transition, having an oncogenic influence in numerous human cancers [18].

The essential role played by the ERK pathway in cancer, as well as the lack of researches about the relation between these novel ERK pathway effectors, and hoping to clarify their role in BC has encouraged us to perform this study. Therefore, the goal of the present study was to define the clinical significance and both the diagnostic and prognostic values of CNK1, Ephrin B1, GPR19 and SMURF1 in Egyptian female BC patients.

2. Experimental

2.1. Subjects

From October 2018 to June 2019, a total of 50 newly diagnosed BC female patients who haven't yet received any chemotherapy or radiotherapy, were recruited from the National Cancer Institute, Cairo University, as well as 15 female patients with chemotherapy resistant BC and 15 female patients with benign breast tumor. The diagnosis was made based on the mammogram and cell biopsy. Moreover, the study involved 10 healthy age and sex-matched volunteers as the healthy control group. All total 65 BC patients (including the chemotherapy resistant group) were subdivided into two groups: metastatic BC (17 patients), and non-metastatic BC group (48 patients).

Peripheral blood samples were withdrawn from all participants. Blood was collected on plain vaccutainer tubes for serum separation. Serum samples were divided into several aliquots and stored at -80 °C for subsequent assay.

2.2. Ethical approval

The study was approved by the Ethical Committee of Research, Faculty of Pharmacy, Ain Shams University (188) and by the Ethical Committee of National Cancer Institute, Cairo University. Additionally, the study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki. Informed consent was obtained from every patient.

2.3. Method

Serum levels of CNK1, Ephrin B1, GPR19 and SMURF1 were determined by ELISA assay using commercially available kits: Human E6627Hu, E6628Hu, E6629Hu and E6630Hu respectively from Bioassay Technology Laboratory (Shanghai, China), as well as determination of human breast cancer antigen CA15.3 and human Carcinoembryonic antigen CEA using commercially available kits from Immunospec (Livonia, USA). All ELISA procedures were done by ELISA chromate micro-plate reader (Awareness technology, USA) according to the manufacturer's instructions.

Table 1: Demographic data and clinical characteristics of the studied groups

Characteristics	Control group	Benign breast tumor group	Malignant breast cancer group				chemotherapy resistant breast cancer group
			[ER$^-$PR$^-$ Her2$^+$]	[ER$^+$PR$^+$Her2$^+$]	[ER$^-$PR$^-$Her2$^-$]	[ER$^+$PR$^+$Her2$^-$]	
Number	10	15	6	11	8	25	15
Age (years) Φ	42.1 ± 11.57	48.19 ± 6.86	57.50 ±6.69	46.64 ± 9.63	52.89 ± 11.40	46.85 ± 10.30	48.20 ±6.37
Chemotherapy	No	No	No	No	No	No	Yes
BC type							
Ductal Carcinoma	–	–	5	10	7	19	11
Lobular Carcinoma	–	–	–	–	–	1	3
Other types	_	_	1	1	1	5	1
Grade I and II	-	-	5	11	6	23	12
III and IV	_	_	1	_	2	2	_
Unidentified	_	_	–	–	–	_	3
Other cancers	_	_	–	–	–	–	–
Hepatitis	0	0	0	0	0	0	0
Thyroid dysfunction	0	0	0	0	0	0	0
CNK1 (ng/L)	39.01 (29.44 – 67.71)	53.46 (37.47 – 62.89)	165.6 (147.3 - 188.5)$^{(a,b)*}$	176.6 (151.1 - 191.7)$^{(a,b)***}$	197.3 (170.4 - 217.0) $^{(a,b)***}$	146.0 (123.6 - 159.8)$^{(a,b,c)*}$	234.8 (200.3 - 269.0)$^{(a,b)***}$
EphrinB1 (ng/ml)	0.51 (0.44-0.78)	0.92 (0.77-1.35)	2.74 (2.01 -3.69)^{a*}	2.23 (1.23-3.74)^{a**}	3.05 (2.42 - 4.08)^{a***b**}	2.52 (1.71 - 3.73)^{a***b**}	3.80 (3.75-4.74)$^{(a,b)***}$
GPR19 (ng/ml)	0.71 (0.47-0.77)	0.62 (0.54-0.67)	1.28 (1.22 - 1.54)^{a**b***}	1.19 (1.01-1.29)^{a*b**}	1.34 (1.10 - 1.52)^{a**b***}	1.07 (0.98 - 1.22)^{a*b**c**}	1.53 (1.35-2.25)$^{(a,b)***}$
SMURF1 (ng/L)	158.5 (103.9-197.7)	193.3 (152.2-235.8)	513.2 (305.3 - 722.2)^{a*}	423.6 (226.4-600.7)^{a*}	673.7 (639.4 - 719.8)$^{(a,b)***}$	514.8 (398.5 - 603.9)$^{(a,b)**}$	681.0 (653.0-740.6)$^{(a,b)***}$

BC: Breast cancer, ER: Estrogen receptor, PR: Progesterone receptor, Her2: Human epidermal growth factor receptor 2
Data are median (25th and 75th centiles-quartiles), Φ = mean ± S.D
a: Significantly different as compared with control group
b: Significantly different as compared with benign group
c: Significantly different as compared with chemotherapy resistant group
*: P<0.05, **: P< 0.01, ***: P< 0.001.

2.4. Statistical analysis

GraphPad Prism 8.0.2 (GraphPad Software, CA, USA) was used for data analysis. Data was expressed as median and IQR. for nonparametric data, Mann–Whitney U test was used for comparison of two independent groups. However, for comparison between more than 2 groups for nonparametric data Kruskall Wallis test was used. Sensitivity and specificity were calculated using the ROC curve and cut off value by SPSS software version 26.0 (SPSS Inc. Chicago, IL, USA). Spearman's ranked correlation test was used to study the possible association between each two variables among each group for nonparametric data. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for association analysis by logistic regression. The probability of error was considered significant at 0.05, while at 0.01 and 0.001 was considered highly significant.

3. Results

3.1. Clinical characteristics of the patients

The clinical and demographic data of the studied groups are summarized in Table 1.

3.2. Serum levels of CNK1, Ephrin B1, GPR19 and SMURF1 in different studied groups

Significantly higher serum levels of CNK1, Ephrin B1, GPR19 and SMURF1 were found in all malignant groups (ER-PR-Her2+, ER+PR+Her2+, ER-PR-HER2-, ER+PR+Her2-) as well as the chemotherapy resistant BC group as compared with the control group. On the other hand, no significant difference was found concerning the benign group as compared to the control.

Moreover, both malignant groups (ER-PR-HER2- and ER+PR+Her2-) as well as the chemotherapy resistant BC group showed significantly higher CNK1, Ephrin B1, GPR19 and SMURF1 serum

levels as compared with the benign group. Nevertheless, the malignant groups ER-PR-Her2+ and ER+PR+Her2+ showed significantly elevated CNK1 and GPR19 serum levels as compared with the benign group, while their serum levels of Ephrin B1 and SMURF1 did not show any significant difference as compared to the benign group.

Additionally, serum levels of CNK1 and GPR19 were significantly higher in the chemotherapy resistant BC group when compared with ER+PR+Her2- group at P<0.01.

To sum up, Fig 1 shows varieties in significant differences for all biomarkers between different studied groups.

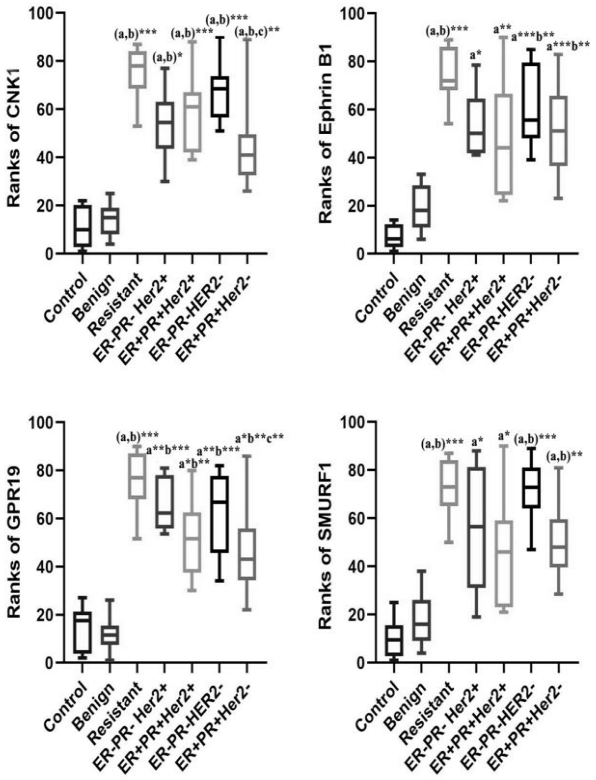

Fig 1 Box plots illustrating serum concentrations of CNK1, Ephrin B1, GPR19 and SMURF1 in different studied groups. Box represents the interquartile range. Line inside the box represents the median. Bars represent minimum and maximum values.
a: Significantly different as compared with control group
b: Significantly different as compared with benign group
c: Significantly different as compared with chemotherapy resistant group
*: P <0.05, **: P < 0.01, ***: P < 0.001

3.3. Serum CNK1, Ephrin B1, GPR19 and SMURF1 levels among metastatic and non-metastatic BC groups

Serum levels of CNK1, Ephrin B1, GPR19 and SMURF1 were all highly significantly elevated in the metastatic BC group when compared to the non-metastatic group at P<0.001 (Fig 2).

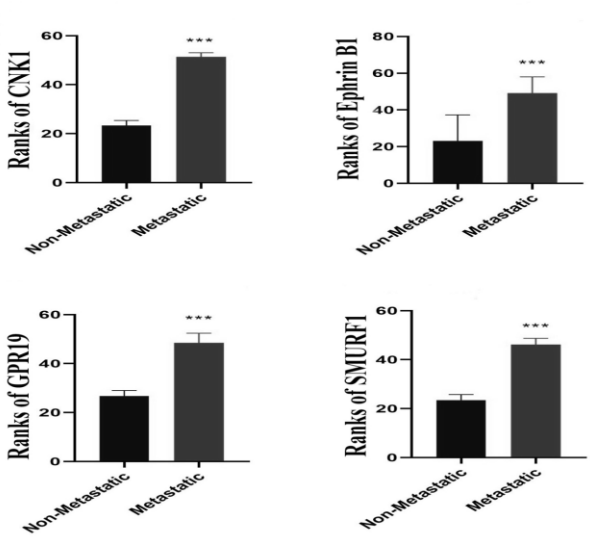

Fig 2 Serum concentrations of CNK1, Ephrin B1, GPR19 and SMURF1 in metastatic and non-metastatic groups. ***: P<0.001

3.4. Correlation analysis of CNK1, Ephrin B1, GPR19 and SMURF1 serum levels in studied groups

The serum level of CNK1 showed a highly significant positive correlation with Ephrin B1, GPR19 and SMURF1 serum levels (r = 0.58, 0.70, 0.61 respectively, P<0.001) in BC patients. Meanwhile, Ephrin B1 was found to be highly significantly positively correlated with GPR19 and SMURF1 (r = 0.59, 0.61 respectively, P<0.001). Both GPR19 and SMURF1 levels also showed a highly significantly positive correlation (r = 0.56, P<0.001). Finally, all biomarkers showed a positive correlation with established tumor markers CA15.3 and CEA (Table 2).

Table 2: Correlation coefficients (r) of CNK1, Ephrin B1, GPR19, SMURF1, CA15.3 and CEA in breast cancer patients

Variable	CNK1	Ephrin B1	GPR19	SMURF1	CA15.3	CEA
CNK1	-	**0.58*** **	**0.70*** **	**0.61*** **	**0.49*** **	**0.32** **
EphrinB1	**0.58*** **	-	**0.59*** **	**0.61*** **	**0.38** **	**0.25***
GPR19	**0.70*** **	**0.59*** **	-	**0.56*** **	**0.58*** **	**0.26***
SMURF1	**0.61*** **	**0.61*** **	**0.56*** **	-	**0.46*** **	**0.36** **
CA15.3	**0.49*** **	**0.38** **	**0.58*** **	**0.46*** **	-	**0.25***

r = Spearman's rho

*** Correlation is significant at the 0.001 level (2-tailed)

** Correlation is significant at the 0. 01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

3.5. The diagnostic value of CNK1, Ephrin B1, GPR19 and SMURF1 serum levels in BC patients

The significance of CNK1, Ephrin B1, GPR19 and SMURF1 serum levels as potential diagnostic biomarkers for de novo BC were assessed using ROC curve. All markers: CNK1 (AUC 0.92; 95% CI 0.85-0.98, P <0.0001), Ephrin B1 (AUC 0.97; 95% CI 0.94-1.00, P <0.0001), GPR19 (AUC 0.86; 95% CI 0.77-0.95, P= 0.0002) and SMURF1 (AUC 0.92; 95% CI 0.86 -0.98, P <0.0001) showed excellent diagnostic value with 100% specificity and 81.54%, 93.85%, 73.85% and 76.92% sensitivity for CNK1, Ephrin B1, GPR19 and SMURF1 respectively. Additionally, the combination of each marker with CA15.3 or CEA revealed improved diagnostic accuracy with improved AUC, sensitivity and specificity (Table 3).

Interestingly, ROC curve for discrimination between metastatic and non-metastatic BC cases showed good diagnostic value using serum levels of CNK1 (ACU 0.87; 95% CI 0.76-0.97, P <0.0001), Ephrin B1 (AUC 0.87; 95% CI 0.79-0.96, P <0.0001), GPR19 (AUC 0.82; 95% CI 0.69-0.94, P <0.0001) and SMURF1 (AUC 0.79; 95% CI 0.69-0.90, P= 0.0003). Most impressively, sensitivity was 70.59% at specificity 91.67% and 81.25% for CNK1, and GPR19 respectively. Sensitivities were 88.24% and 82.35% at specificities 75% and 66.67%, respectively for Ephrin B1 and SMURF1 (Fig 3).

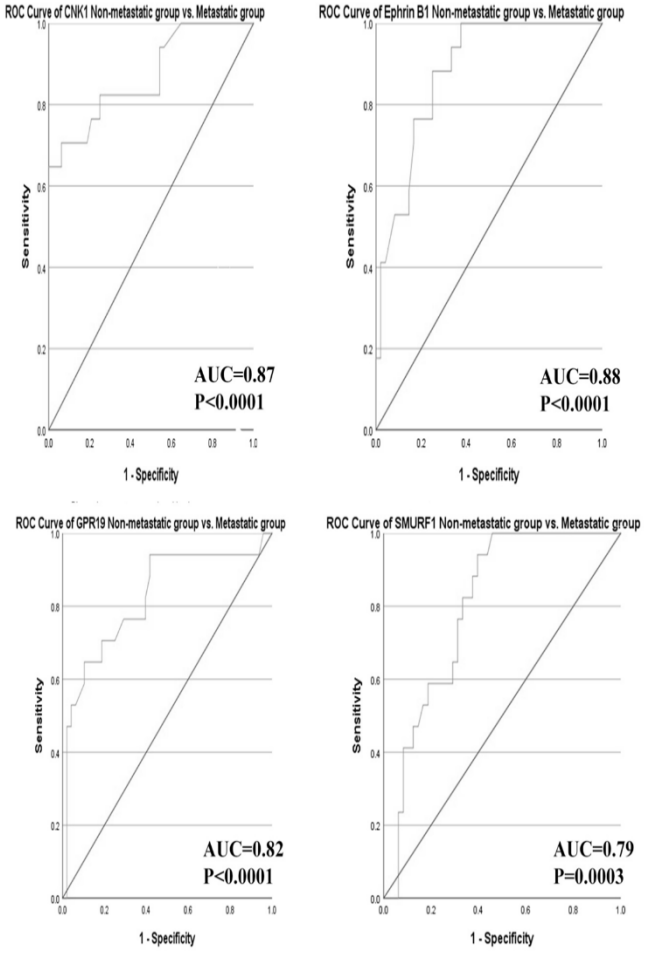

Fig 3 Receiver Operator Characteristics (ROC) curve for discrimination between metastatic and non-metastatic breast cancer cases using CNK1, Ephrin B1, GPR19 and SMURF1 serum levels

Table 3: Receiver Operator Characteristics (ROC) curve for evaluating the diagnostic value of CNK1, Ephrin B1, GPR19, SMURF1, CA15.3 and CEA serum levels for breast cancer

Variable(s)	AUC	P-value	95% Confidence Interval		Cut-off value	Sensitivity (%)	Specificity (%)
			Lower Bound	Upper Bound			
CA15.3 serum level	**0.93**	**<0.0001**	**0.86**	**0.99**	**>67.89 U/ml**	**89.2**	**90.0**
CEA serum level	**0.90**	**<0.0001**	**0.83**	**0.98**	**>0.64 ng/ml**	**90.8**	**80.0**
CNK1serum level	**0.92**	**<0.0001**	**0.85**	**0.98**	**>74.33 ng/L**	**81.54**	**100.0**
(CNK1 + CA15.3)	**0.96**	**<0.0001**	**0.91**	**1.00**	**> 0.79**	**90.8**	**90.0**
(CNK1+ CEA)	**0.95**	**<0.0001**	**0.91**	**1.00**	**> 0.90**	**89.2**	**100.0**
Ephrin B1 serum level	**0.97**	**<0.0001**	**0.94**	**1.00**	**>0.83 ng/ml**	**93.85**	**100.0**
(Ephrin B1+ CA15.3)	**0.99**	**<0.0001**	**0.98**	**1.00**	**> 0.67**	**98.5**	**100.0**
(Ephrin B1+ CEA)	**0.98**	**<0.0001**	**0.96**	**1.00**	**> 0.69**	**95.4**	**100.0**
GPR19 serum level	**0.86**	**0.0002**	**0.77**	**0.95**	**>0.89 ng/ml**	**73.85**	**100.0**
(GPR19 + CA15.3)	**0.96**	**<0.0001**	**0.91**	**1.00**	**> 0.72**	**92.3**	**90.0**
(GPR19 + CEA)	**0.93**	**<0.0001**	**0.87**	**0.99**	**> 90**	**80.0**	**100.0**
SMURF1 serum level	**0.92**	**<0.0001**	**0.86**	**0.98**	**>232.6 ng/L**	**76.92**	**100.0**
(SMURF1 + CA15.3)	**0.97**	**<0.0001**	**0.92**	**1.00**	**> 0.76**	**92.3**	**90.0**
(SMURF1 + CEA)	**0.95**	**<0.0001**	**0.91**	**1.00**	**> 0.90**	**84.6**	**100.0**
(CNK1+Ephrin B1+GPR19+SMURF1+CA15.3)	**0.99**	**<0.0001**	**0.98**	**1.00**	**> 0.85**	**96.9**	**100.0**
(CNK1+Ephrin B1+GPR19+SMURF1+CEA)	**1.00**	**<0.0001**	**1.00**	**1.00**	**> 0.49**	**100.0**	**100.0**

3.6. Independent factors for BC metastasis risk prediction

Logistic regression was performed to detect independent predicting factors significantly associated with BC metastasis risk. This analysis revealed that CNK1 (OR = 1.025, 95% CI = 1.004-1.047, P 0.021) and Ephrin B1 (OR = 6.574, 95% CI = 1.139-37.957, P 0.035) could be considered as independent predictors of BC metastasis risk.

4. Discussion

BC is the most prevalently detected cancer among women and causes significant mortality throughout the world [19]. The need of the hour is to find out efficient diagnostic and prognostic markers for BC. Thus, this study was designed to evaluate the usefulness of CNK1, Ephrin B1, GPR19 and SMURF1 serum levels as novel biomarkers for BC diagnosis and to improve the sensitivity of BC detection by their combination with the established

tumor markers CA 15.3 or CEA, as well as to predict BC metastasis risk in Egyptian female BC patients.

The ERK signaling pathway is along with the main mechanisms that transmits signals from the cell surface to the nucleus. ERK is a very well conserved serine/threonine kinase activated by MEK through phosphorylation of both tyrosine and threonine residues. This pathway is involved in G1 cell cycle progression [20]. Thus, it is not surprising that several components that activate this pathway are involved in carcinogenesis [21].

The current study discusses recent findings, concerned with ERK signaling pathway effectors that mediate malignant transformation. Several moderators of ERK signaling, such as CNK1, Ephrin B1, GPR19 and SMURF1, could control the duration and extent of ERK activity and thus may play role in controlling cell cycle or carcinogenesis [22]. Several ERK pathway effectors were found to be up-regulated in several cancers, including BC [23, 24], Renal cell carcinoma [25], colorectal cancer [26], pancreatic cancer [27] and lung cancer [28].

Accordingly, our present study demonstrated that CNK1, Ephrin B1, GPR19 and SMURF1 serum levels were significantly higher in BC patients and revealed excellent diagnostic accuracy for de novo BC which was even improved when combined with the established tumor markers CA15.3 or CEA. Additionally, the significant positive correlations found between each pair of these biomarkers reveal their synergistic role as positive regulators of the ERK signaling pathway.

Previous studies described CNK1 as an important promoter of tumorigenesis, driving oncogenic pathways in cancer cells [14, 29]. CNK1 activates the ERK pathway through mediating Src-dependent tyrosine phosphorylation and activation of the Raf-1/ERK pathway cascade [30, 31]. An earlier study by Fritz et al. demonstrated that CNK1 was highly expressed in carcinoma in situ and invasive BC tumors compared with normal breast tissue sections, and that CNK1 was chiefly localized at the plasma membrane of BC cells, regulating anchorage-independent proliferation and emphasizing the development of BC cells [32].

Furthermore, CNK1/Ephrin B1 interaction was found necessary for the activation of the Ephrin B1-associated MAPK/ERK pathway, thus playing together a key role in cell survival, malignant transformation and cancer development [33]. The Eph family of receptor tyrosine kinases together with their corresponding ephrin ligands are cell-signaling membrane-bound proteins that have crucial governing roles in cancer progression. Ephrin B1 is a type I membrane protein and a ligand of Ephrin-related receptor tyrosine kinase [34, 35].

Interestingly, our study revealed significantly higher CNK-1 and Ephrin B1 serum levels in the chemotherapy resistant BC patients as well as the metastatic BC group other than the non-resistant and non-metastatic groups respectively. This confirms their crucial role in proliferation, survival, differentiation, and migration [32]. In agreement with our data, it was recently reported that high expression of Ephrin B1 was positively correlated with metastasis in BC [36, 37]. Moreover, a previous study showed that downregulation of CNK1 reduces the cancer cells' invasiveness and correlates with matrix metalloproteinases' diminished expression [14, 29]. Accordingly, it seems from the present findings that CNK1/Ephrin B1 interaction might play a fundamental role as a mediator of oncogenic signaling that promotes BC resistance, eventually leading to invasion and metastasis.

Another potential regulator of the ERK pathway is GPR19, a member of G protein-coupled receptors (GPCRs) family. Dysregulation of GPCR signaling members has been documented as a mediator of cancer initiation and progression [38]. Moreover, GPR19 is tightly associated with the malignant transformation of mammary cells [39], thus explaining its significant elevation in the BC patients as compared to the healthy control group. The study of O'hayre et al. demonstrated that abnormal expression of GPR19 induces continual unrestrained cell migration, activates intracellular signal transduction and eventually causes cancer cells growth, stimulating angiogenesis and metastasis [40]. Likewise, Rao et al. provided evidence that GPR19 plays a potential role in metastasis by promoting the mesenchymal-epithelial transition through the MAPK/ERK pathway, thus facilitating colonization of metastatic breast tumor cells. Accordingly, our results confirmed this uncontrolled proliferation and high metastatic abilities as a significant elevation of GPR19 was demonstrated in the metastatic group compared with the non-metastatic group [16].

As previously mentioned, activation of ERK pathway plays a fundamental role in cell proliferation, differentiation, migration, senescence and apoptosis [41]. The study of Sun et al. demonstrated that induction of the MAPK/ERK pathway up-regulates the expression of SMURF1, a well-known E3 ubiquitin ligase that targets substrate proteins for ubiquitination and proteasomal degradation [42]. Amassing studies have revealed that SMURF1 acts as an oncogenic factor in several human cancers such as gastric cancer [43], lung cancer [44] and renal cell carcinoma [45]. Similarly in the present study, up-regulated serum level of SMURF1 was demonstrated in BC patients especially in the chemotherapy resistant group, revealing that

high levels of SMURF1 may be associated with drug resistance. Interestingly the study of Kwei et al. identified SMURF1 as an augmented oncogene leading to cell invasiveness in pancreatic cancer [46]. We correspondingly demonstrated higher SMURF1 levels in the metastatic BC group, proving that SMURF1 may be associated with cell invasion and migration in BC.

To summarize, the current study demonstrated that CNK1, Ephrin B1, GPR19 and SMURF1 serum levels may be novel diagnostic and prognostic biomarkers for BC and may be of value in metastasis prediction that deserves consideration by further validation studies.

5. Conclusion

In conclusion, our study highlights the synergistic role of CNK1, Ephrin B1, GPR19 and SMURF1 in development and progression of BC as well as their potential role in cancer invasion and metastasis. Future studies and investigations are still warranted to prove their possible utility as future therapeutic targets.

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7. Conflicts of interest

No potential conflict of interest was reported.

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