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Identifying the role of PTPN12 expression in predicting the efficacy of capecitabine to neoadjuvant chemotherapy in breast cancer treatment

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Abstract. – OBJECTIVE: The aim of this study was to investigate the expression of PTPN12 in human breast cancer and its role in predicting the efficacy of neoadjuvant chemotherapy (NACT) for breast cancer.

PATIENTS AND METHODS: The PTPN12 expression levels were assessed by immunohistochemical staining in 114 breast cancer patients. The correlation of PTPN12 with clinicopathological features was also analyzed. Multivariate logistic regression was used to explore the effect of PTPN12 expression in predicting clinical response.

RESULTS: We observed a significant association of PTPN12 expression with cTNM classification. The overall pathological complete response (pCR) rate was 23.2 % in high PTPN12 expression group, whereas it was 5.2% in low PTPN12 expression group. The multivariate regression analyses further indicated that clinical response correlated with PTPN12 expression level and cycles of NACT, and CEX regimen was associated with the overall pathological complete response. In addition, Spearman rank correlation analyses suggested that higher PTPN12 expression indicated better clinical response in breast cancer patients. Furthermore, PTPN12 expression statistically related with pathological response in TNBC and Luminal B subtypes, as assessed by Pearson Chi-square test or Fisher's exact test.

CONCLUSIONS: Our study informed that cTNM classification is an independent risk factor for PTPN12 expression and PTPN12 is an independent predictor to clinical remission.

Key Words
Breast cancer, PTPN12, Predictive factor, Capecitabine, Immunohistochemistry.

Introduction

Based on the GLOBOCAN 2012 database, breast cancer with 22.1% incidence rate has been the most frequent female malignancy in China and jeopardized the women's health and survival[1]. Although the improvement in early screening methods to detect breast cancer has led to decrease in the morbidity of these patients, there are still some patients who are diagnosed with locally advanced breast cancer, and contribute to the overall poor prognosis with 5-year survival rate less than 50%[2]. Breast cancer is an intra-heterogeneous disease depicting many pathological and biological characteristics, which has not been properly described and classified by known biomarkers. These limitations challenge the individualized treatment and prognosis[3].

Neoadjuvant chemotherapy (NACT) is performed with an intention to downstage the breast cancer, and improve the chances of the surgical option, in addition to acquiring early information about the response to chemotherapy and the biological features of the disease[4]. These multiple advantages of NACT to breast cancer patients make it an attractive option. Several large, randomized clinical trials have shown that there is no significant survival difference between NACT and adjuvant chemotherapy[5,6]. The systemic management of breast cancer has led to the application of neoadjuvant chemotherapy in not only for locally advanced breast cancer, but also in patients with T1N1M0, T2N0M0, T3N0M0 and T2N1M0 stages, classified based on cTNM

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staging system, designed by AJCC (American Joint Committee on Cancer). Pathological complete response (pCR), a predictor of overall survival (OS) and disease-free survival (DFS), is believed to be affected by multiple factors including, molecular subtypes, regimens, curative cycles, biomarkers and even the different pCR definitions[7]. In addition to ER, PR, HER2 and Ki67 biomarkers, the PIK3CA, β3-tubulin and CCND1 and other biomarkers have also been suggested to be NACT predictors. But, there are still many controversies associated with it because of the inconsistent conclusions and limited researchments[8-11]. Currently, NACT regimens are mainly based on anthracycline and taxane drugs[12,13]. Capecitabine, an oral fluoropyrimidine agent, affect DNA replication and repair by modifying the thymidylate synthase, and its combination with other agents, such as docetaxel, platinum increased the clinical and pathological remission rate and shown a significant survival benefit for cancer patients[14-16]. Earlier reports have suggested that capecitabine could be a promising agent in NACT and deserved further investigation.

Protein tyrosine phosphatase non-receptor type 12 (PTPN12), a member of protein tyrosine phosphatases (PTPs), is a widely expressed mammalian cytoplasm protein that has been detected to be related to several carcinomas. It has been linked to the regulation of cell growth, adhesion, epithelial mesenchymal transition (EMT), migration and invasiveness[17,18]. PTPN12 has been suspected to be a tumor suppressor in triple-negative breast cancer by inhibiting receptor tyrosine kinases (RTKs)[19]. And PTPN12 is a protective prognosis factor for breast cancer[20]

Consistent with the role of NACT in breast cancer patients, it is important to identify novel biomarkers that can act as predictors for this regimen selection and clinical pathological remission. In addition, the use of new regimens and drug combinations as a part of NACT may lead to improved therapeutic effects. Thus, in this study, we have also tried to understand the role and link of PTPN12 as a biomarker in deciphering the sensitivity and curative effect of new neoadjuvant chemotherapies, including cyclophosphamide, epirubicin and capecitabine (CEX). We have specifically explored the relationship between PTPN12 expression and clinical pathological characteristics in breast cancer patients treated with NACT regimens including CEX regimen.

Patients and Methods

Patients and tissue samples

The study was approved by the Medical Science Research Ethics Committee of First Affiliated Hospital of China Medical University, Shenyang, China, and informed consent (written/verbal) was obtained from all the enrolled patients. This retrospective study is based on the analysis of 114 female primary breast cancer patients, who were histologically diagnosed with breast cancer in the First Affiliated Hospital, China Medical University between September 2011 and December 2014. The samples were selected based on the following inclusion criteria: a) pathologically confirmation of infiltrative ductal carcinoma by core needle biopsy or incisional biopsy; b) no distant metastasis detected according to the MRI, CT or ultrasound before NACT; c) patients have not received any previous chemotherapy, endocrine therapy, radiotherapy or target therapy; d) the status of ER, PR, HER2 and KI67 was known; e) paraffin-embedded biopsy tissues were available; and f) all patients have accepted post-NACT surgical treatment. The median age of the selected patients was 50 years at diagnosis, ranging from 25 to 69. According to the clinical staging criteria set by the American Joint Committee on Cancer, 39 patients were at stage II, while 75 were at stage III.

Immunohistochemistry

The formalin-fixed, paraffin-embedded breast cancer tissues were cut into 4 μm sections and were stained using immunohistochemical streptavidin-peroxidase (S-P) method. The sections were first dewaxed, rehydrated, and then underwent high pressure antigen retrieval for 2 minutes in a citrate buffer (pH=6.0). Ultra-sensitive™ S-P Kit (Maixin-Bio, Fuzhou, China) was used based on the reagent manual to block endogenous peroxidase activity and reduce non-specific reactivity. The sections were incubated with primary antibody against PTPN12 (Abcam, ab154892, 1:800 dilution, Abcam, Cambridge, UK) at 4°C for overnight. Later, the sections were incubated with secondary antibody and streptomycin avidin-peroxidase, successively using Ultra-sensitive™ S-P kit, and finally visualized with DAB reagent. For the negative control, the primary antibody was replaced by PBS.

Neoadjuvant chemotherapy (NACT) regimens

Three different NACT regimens were administered as follows: CEF (cyclophosphamide 1000 mg, epirubicin 80 mg/m^2, and 5-fluorouracil

750 mg, every 3 weeks), TEC (docetaxel 75 mg/m^2, epirubicin 80 mg/m^2, and cyclophosphamide 1000 mg, every 3 weeks), and CEX (cyclophosphamide 1000 mg, epirubicin 80 mg/m^2 every 3 weeks, capecitabine 1250 mg/m^2/day twice daily for 2 weeks). The subjects received NACT for a median of 4 cycles (range 1-6 cycles) before surgery. Of the 114 eligible patients, 42 received CEX, 17 received TEC and 55 received CEF neoadjuvant chemotherapy regimens.

Evaluation of NACT response

The clinical response to NACT was evaluated by physical and imaging examinations of the patients, according to Response Evaluation Criteria in Solid Tumors (RECIST1.0). Breast primary carcinoma was regarded as a target lesion. The baseline, which is usually the sum of axes, was calculated as the sum of the long diameters of primary breast tumors. Complete Response (CR) represented disappearance of all target lesions. Partial Response (PR) represented the sum of axes achieved at least a 30% decrease from the baseline. Stable Disease (SD) represented the sum of axes variations between PR and PD. Progressive Disease (PD) represented the sum of axes that achieved at least 20% increase on the baseline or emergence of new lesions. A pCR was defined as the absence of invasive breast cancer cells in breast and nodes (ypT0/Tis ypN0). Patients who had a CR or PR were analyzed together as an efficacy group, whereas patients with a SD or PD were part of inefficacy group. The primary endpoint was the objective clinical response after NACT, while the secondary endpoint was a pathological complete response.

Evaluation of immunohistochemical staining

Two professional pathologists evaluated the whole sections independently. PTPN12 expression was detected in the cytoplasm. Expression of PTPN12 was estimated by double score semi-quantitative analysis, namely the staining intensity and the percentage of positive cells. Staining intensity was recorded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). As for the percentage of positive cells, scores were marked as follow, 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%). The final immunohistochemical staining score was determined by multiplying the staining intensity levels with the positive percentage staining scores. All patients were subsequently divided into two groups, according to the immunohistochemical staining scores: low expression (score $\leq$ 3), high expression (score > 3).

Statistical Analysis

Statistical analyses were performed using the SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). The data were analyzed by applying Pearson chi-square analysis, Fisher's exact test, logistic regression analyses and Spearman rank correlation. All the statistical tests were two-sided, and a p-value of <0.05 was considered statistically significant.

Results

Analysis of PTPN12 expression and its association with clinical pathologic parameters of breast cancer patients

Immunohistochemical staining of PTPN12 in invasive breast carcinoma and benign breast tumor was performed, and we observed a wide range of staining starting from negative to moderate to high, as shown in Figure 1 A-E. The clinical pathologic characteristics of patients are shown in Table I. Successively, based on Pearson Chi-square or Fisher's exact tests, we evaluated the relationship between clinical pathological parameters and PTPN12 expression. Our data indicated that PTPN12 expression statistically correlated with cTNM classification, and primary site HER2 status, respectively (χ^2 = 7.300, p = 0.007; χ^2 = 4.567, p = 0.033). Nevertheless, no statistically significant correlation was observed with age at diagnosis, clinical T stage, clinical N stage, histological grade, and ER/PR/Ki67 status in the primary site. The cTNM classification also appeared to be an independent factor correlated with PTPN12 expression by multivariate logistic regression analysis (p = 0.002), as shown in Table II.

Correlation between clinical pathological parameters and NACT treatment response

Clinical pathological parameters of 114 enrolled patients were aligned with the clinical response to NACT treatment and pathological response, separately, by univariate logistic regression analyses, as shown in Table III. The clinical response revealed statistically significant correlation with HER2 status in primary site (p = 0.016), Ki67 status in primary site (p = 0.020), molecular subtypes (p = 0.046), NACT regimens

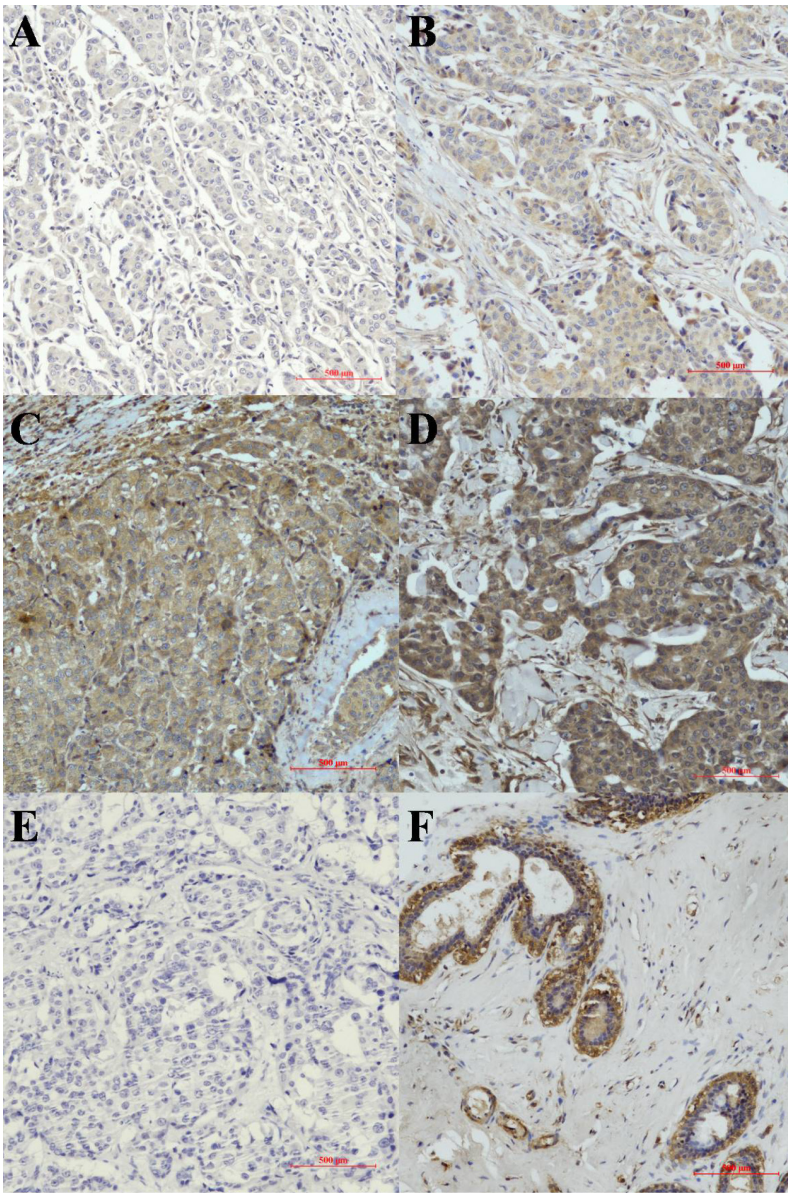

Figure 1. Analysis of PTPN12 expression level in different breast tissues. Immunohistochemical staining of tyrosine-protein phosphatase non-receptor type 12 (PTPN12) in invasive breast carcinoma and benign breast tumor: (**A**-**B**) Negative and weak staining intensity representing the low PTPN12 expression in breast carcinoma tissue; (**C**-**D**) Moderate and strong staining intensity representing the high PTPN12 expression in breast carcinoma tissue; (**E**) Negative control; (**F**) Positive control. The yellow or brown color cytoplasm represents PTPN12 expression, while the blue color represents nuclei. The bar scale represents 500 μM.

(p = 0.037), cycles of NACT (p = 0.007) and PTPN12 expression (p<0.001). In addition, we also observed statistically significant relationship between pathological response and cTNM classification (p = 0.01), along with Ki67 status in primary site (p = 0.040), NACT regimens (p < 0.001), and PTPN12 expression (p = 0.006). Multivariate logistic regression analyses, as shown Table IV, illustrated that PTPN 12 expression level and cycles of NACT were positively correlated with NACT clinical response (p = 0.001; p = 0.030 respectively). Furthermore, NACT regimen appeared to be an independent factor in pathological response to NACT treatment (p = 0.014, Table V).

Analysis of NACT treatment response and PTPN12 expression

The overall relationship between PTPN12 expression and the response to NACT treatment has been shown in Table VI. PTPN12 expression level was significantly associated with clinical and pathological response, as confirmed by Pearson Chi-square or Fisher's exact test (χ^2 = 13.031, p = 0.001; p = 0.006) respectively. Further analysis of PTPN12 correlation, based on its immunohistochemical staining score of 0-12, with clinical response as defined by PD, SD, PR and CR, again revealed statistically significant positive correlation as demonstrated by Spearman rank correlation test (p = 0.009).

Table I. Association of PTPN12 expression level with clinicopathological parameters.

Factor	Total (%)	PTPN12 expression		*p*-value	χ^2 ($p \leq 0.05$)
		Low (n=58, 50.9%)	High (n=56, 49.1%)		
Age at diagnosis (years)				0.833[a]	
Median	50	50	50		
Range	25-69	25-69	31-66		
Clinical T stage				0.459[b]	-
T1	4 (100.0)	2 (50.0)	2 (50.0)		
T2	39 (100.0)	16 (41.0)	23 (59.0)		
T3	34 (100.0)	20 (58.8)	14 (41.2)		
T4	37 (100.0)	20 (54.1)	17 (45.9)		
Clinical N stage				0.649[a]	
N0	23 (100.0)	9 (39.1)	14 (60.9)		
N1	53 (100.0)	28 (52.8)	25 (47.2)		
N2	11 (100.0)	6 (54.5)	5 (45.5)		
N3	27 (100.0)	15 (55.6)	12 (44.4)		
cTNM classification				0.007[a]	7.3
II	39 (100.0)	13 (33.3)	26 (66.7)		
III	75 (100.0)	45 (60.0)	30 (40.0)		
Histologic grade				0.763[a]	
II	91 (100.0)	47 (51.6)	44 (48.4)		
III	10 (100.0)	4 (40.0)	6 (60.0)		
Unclassified	13 (100.0)	7 (58.3)	6 (46.2)		
ER status in primary site				0.174[a]	
Negative	48 (100.0)	28 (58.3)	20 (41.7)		
Positive	66 (100.0)	30 (45.5)	36 (54.5)		
PR status in primary site				0.265[a]	
Negative	61 (100.0)	34 (55.7)	27 (44.3)		
Positive	53 (100.0)	24 (45.3)	29 (54.7)		
HER2 status in primary site				0.033[a]	4.567
No amplification	46 (100.0)	29 (63.0)	17 (37.0)		
Amplification	68 (100.0)	29 (42.6)	39 (57.4)		
Ki67 status in primary site				0.525[a]	
<14%	21 (100.0)	12 (57.1)	9 (42.9)		
≥14%	93 (100.0)	46 (49.5)	47 (50.5)		
Molecular subtype				0.033[b]	-
Luminal A	8 (100.0)	5 (62.5)	3 (37.5)		
Luminal B	60 (100.0)	27 (45.0)	33 (55.0)		
HER2-enriched	22 (100.0)	8 (36.4)	14 (63.6)		
TNBC[c]	24 (100.0)	18 (75.0)	6 (25.0)		

[a]Pearson Chi-square, [b]Fisher's Exact test, [c]Triple-negative breast cancer.

Analysis of the role of PTPN12 in guiding the selection of NACT regimens

We observed a statistically significant association between NACT regimens and the clinical response to NACT among patients with low PTPN12 expression (p = 0.016), as shown in Table VII. Among these 9 (69.2%) cases acquired clinical remission in the CEX group, while 25 (75.8%) suffered clinical progression in the CEF group. In contrast, no parallel association was observed in the high PTPN12 expression group (data not shown).

Table II. Multivariate logistic regression analysis to predict correlation of PTPN12 expression.

Factor	PTPN12 expression		
	p-value	OR	95%CI
cTNM classification			
II vs. III	0.002	0.230	0.091-0.581

Table III. Univariate analyses to predict clinical response and pathological response

	Clinical response				Pathological response		
	Total (%)	SD/PD[a] (n=50)	CR/PR[b] (n=64)	*p*-value	no pCR[c]	yes pCR[d] (n=98)	*p*-value (n=16)
Age at diagnosis (years)				0.671			0.965
≤49	55 (48.2)	23 (46.0)	32 (50.0)		49 (89.1)	6 (10.9)	
≥50	59 (51.8)	27 (54.0)	32 (50.0)		49 (83.1)	10 (16.9)	
Clinical T stage				0.219			0.256
T1	4 (3.5)	3 (75.0)	1 (25.0)		3 (75.0)	1 (25.0)	
T2	39 (34.2)	20 (51.3)	19 (48.7)		31 (79.5)	8 (20.5)	
T3	34 (29.8)	15 (44.1)	19 (55.9)		29 (85.3)	5 (14.7)	
T4	37 (32.5)	12 (32.4)	25 (67.6)		35 (94.6)	2 (5.4)	
Clinical N stage				0.69			0.496
N0	23 (20.2)	9 (39.1)	14 (60.9)		18 (78.3)	5 (21.7)	
N1	53 (46.5)	25 (47.2)	28 (52.8)		45 (84.9)	8 (15.1)	
N2	11 (9.6)	6 (54.5)	5 (45.5)		10 (90.9)	1 (9.1)	
N3	27 (23.7)	10 (37.0)	17 (63.0)		25 (92.6)	2 (7.4)	
cTNM classification				0.451			0.01
II	39 (34.2)	19 (48.7)	20 (51.3)		29 (74.4)	10 (25.6)	
III	75 (65.8)	31 (41.3)	44 (58.7)		69 (92.0)	6 (8.0)	
Histologic grade				0.574			0.284
II	91 (79.8)	41 (45.1)	50 (54.9)		76 (83.5)	15 (16.5)	
III	10 (8.8)	5 (50.0)	5 (50.0)		10 (100.0)	0 (0)	
Unclassified	13 (11.4)	4 (30.8)	9 (69.2)		12 (92.3)	1 (7.7)	
ER status in primary site				0.984			0.886
Negative	48 (42.1)	21 (43.8)	27 (56.2)		41 (85.4)	7 (14.6)	
Positive	66 (57.9)	29 (43.9)	37 (56.1)		57 (86.4)	9 (13.6)	
PR status in primary site				0.219			0.762
Negative	61 (53.5)	30 (49.2)	31 (50.8)		53 (86.9)	8 (13.1)	
Positive	53 (46.5)	20 (37.7)	33 (62.3)		45 (84.9)	8 (15.1)	
HER2 status in primary site				0.016			0.057
No amplification	46 (40.3)	26 (56.5)	20 (43.5)		43 (93.5)	3 (6.5)	
Amplification	68 (59.7)	24 (35.3)	44 (64.7)		55 (80.9)	13 (19.1)	
Ki67 status in primary site				0.02			0.04
<14%	21 (18.4)	14 (66.7)	7 (33.3)		21 (100.0)	0 (0)	
≥14%	93 (81.6)	36 (38.7)	57 (61.3)		77 (82.8)	16 (17.2)	
Molecular subtypes				0.046			0.337
Luminal A	8 (7.0)	6 (75.0)	2 (25.0)		8 (100.0)	0 (0)	
Luminal B	60 (52.6)	24 (40.0)	36 (60.0)		51 (85.0)	9 (15.0)	
HER2-enriched	22 (19.3)	6 (27.3)	16 (72.7)		17 (77.3)	5 (22.7)	
TNBC	24 (21.1)	14 (58.3)	10 (41.7)		22 (91.7)	2 (8.3)	
NACT				0.037			<0.001
CEX	42 (36.8)	12 (28.6)	30 (71.4)		28 (66.7)	14 (33.3)	
TEC	17 (14.9)	8 (47.1)	9 (52.9)		17 (100.0)	0 (0)	
CEF	55 (48.2)	30 (54.5)	25 (45.5)		53 (96.4)	2 (3.6)	
Cycle of NACT				0.007			0.101
1-3	50 (43.9)	29 (58.0)	21 (42.0)		46 (92.0)	4 (8.0)	
4-6	64 (56.1)	21 (32.8)	43 (67.2)		52 (81.2)	12 (18.8)	
PTPN12 expression				0.001			0.006
Low	58 (50.9)	35 (60.3)	23 (39.7)		55 (94.8)	3 (5.2)	
High	56 (49.1)	15 (26.8)	41 (73.2)		43 (76.8)	13 (23.2)	

[a]Stable Disease and Progressive Disease, regarded as clinical progression, [b]Complete Response and Partial Response, regarded as clinical remission, [c]Patients did not acquire pathological complete response, [d]Patients acquired pathological complete response.

Table IV. Multivariate logistic regression analyses to predict clinical response.

	Clinical response		
Factor	*p*-value	OR	95%CI
PTPN12 expression			
Low vs. high	0.001	5.547	2.020-15.231
Cycles of NACT			
1-3 vs. 4-6	0.030	2.861	1.107-7.393

Table V. Multivariate logistic regression analysis to predict pathological response.

	Pathological
Factor	*p*-value
NACT regimen	
CEX vs. TEC and CEF	0.014

Analysis of the role of PTPN12 as predictor for pCR in TNBC and Luminal B patients

We observed a statistically improved pCR rate in TNBC group, when PTPN12 was highly expressed (40% *vs.* 0%, $p = 0.036$). However, this pCR rate increased from 3.7% to 24.2%, $p = 0.033$, in the luminal B group patients, as shown in Table VIII.

Discussion

In this study, we observed a statistically significant association of PTPN12 expression with clinical characteristics, specifically cTNM and HER2 status, in patients treated with NACT. Multiple regression analysis confirmed the independent correlation of cTNM with PTPN12 levels. Consistent with our observations, some other studies have also suggested the relationship of PTPN12 with clinicopathological characteristics and outcome[20]. Another study showed that PTPN12 acted as a tumor suppressor in triple-negative breast cancer[19]. Further detailed analysis in our study indicated that in comparison to the overall PTPN12 expression, the ratio of patients in stages T1N1M0, T2N0M0, T3N0M0 and T2N1M0 (66.7%) have significantly higher PTPN12 expression ($p = 0.007$). The multivariate logistic regression analyses revealed a negative correlation between PTPN12 expression and cTNM classification. HER2 amplification, detected at the primary site by IHC or FISH analysis, was not observed in 46 cases, but 29 (63.0%) cases showed low PTPN12 expression. In addition, among the four molecular subtypes, PTPN12 expression rate varied significantly ($p = 0.033$), but to a certain extent was consistent with its relationship with HER2 status. Specifically, in the TNBC subtype, 18 (75.0%) cases were classified as PTPN12 low expression and it was the highest rate compared to other subtypes. The 62.5% of the cases in the luminal A subtype also showed low PTPN12 expression. In contrast, only 8 (36.4%) patients in HER2-enriched subtype have low PTPN12 expression. Consistent with our observations, the previous study has also shown that PTPN12 was undetectable in 37.0% of the invasive breast cancer and 60.4% of the TNBC patients, but the overall tendency of PTPN12 expression among the four molecular subtypes was same as our results[19].

The pathological complete response is generally accepted as a surrogate for a better outcome. Thus, acquiring pCR for all subtypes of breast cancer has been the expectation, as a similar response in HER2-enriched and TNBC subtypes is equivalent to long DFS (disease-free survival) or OS (overall survival)[12,13]. Moreover, due to non-availability of current biomarkers to precisely predict NACT efficacy, our results are a step in that direction, as we observed that PTPN12

Table VI. Correlation between NACT treatment response and PTPN12 expression.

	N(%)	Clinical response		χ^2	*p*	Pathological response		*p*
		SD/PD	CR/PR			No	Yes	
PTPN12	114 (100.0)	50 (43.9)	64 (56.1)			98 (86.0)	16 (14.0)	
Low	58 (50.9)	35 (60.3)	23 (39.7)	13.031	<0.001[a]	55 (94.8)	3 (5.2)	0.006[b]
High	56 (49.1)	15 (26.8)	41 (73.2)			43 (76.8)	13 (23.2)	

[a]Pearson Chi-square, [b]Fisher's Exact test.

Table VII. Correlation between PTPN12 expression and the selection of NACT regimens.

	Low PTPN12 expression				
	N	CR/PR	SD/PD	*p*-value	χ^2
TEC	13	6 (46.2)	7 (53.8)		
CEX	13	9 (69.2)	4 (30.8)	0.016	8.296
CEF	33	8 (24.2)	25 (75.8)		

expression had some relevance in forecasting the efficacy of NACT. So far, molecular subtypes markers have been widely accepted as the most important predictors of NACT with specific clinical pathological characteristics, biological behavior, drug sensitivity and have a history of predictive value[7]. Based on our study, use of PTPN12 expression as an independent prognosis factor can be adapted because high PTPN12 level has been observed to be a significant factor indicating clinical remission (CR/PR *vs.* SD/PD) and pCR of the entire cohort. Spearman rank correlation test suggested that the score of PTPN12 expression is related with NACT response based on RECIST1.0 criteria. Moreover, multivariate analyses also indicated PTPN12 expression ($p = 0.001$) and cycles of NACT ($p = 0.030$) as independent predictors of clinical remission. The enrolled patients who have high PTPN12 expression have the best ontcomes with 4-6 cycles of chemotherapy treatment. They are always accompanied by a better prognosis. The NACT regimen ($p = 0.014$) which is the only independent factor acquiring pCR refers to CEX regimen. However, our study indicated that there was no statistical correlation of pCR with molecular subtypes or PTPN12. The hormone receptors, HER2 status, histological grade did not show any relevance to pCR and in TNBC group, the pCR rate was just 8.3%, far below the previously reported data as mentioned above. This discrepancy might be due to a higher number of cases with low PTPN12 expression in TNBC group. Our findings did not confirm PTPN12 as a predictor of NACT and can be due to the small sample size. Nevertheless, we did observe that PTPN12 may predict clinical remission. Furthermore, on the basis of subgroup analyses, we observed that in the TNBC and luminal B groups, high PTPN12 expression was statistically associated with pCR. TNBC group in itself is a heterogeneous group where luminal AR subgroup has higher frequency mutation of PIK3CA. Thus, the combined medication of PI3K inhibitors and AR inhibitors could improve the pCR rate. In contrast, the luminal B group consists of $HER2^-$ and $HER2^+$ subtypes. Therefore, due to a complex pattern of different markers in these subgroups, require additional recognition of biomarkers to predict the response to NACT and to further improve the optimization selection of NACT regimens.

There has been no ideal regimen as per NACT guideline. But anthracyclines/docetaxel-based regimen has been considered as the most efficacious neoadjuvant chemotherapy and been investigated in some large, randomized clinical trials[12,13]. Some studies[21-23] have indicated that utility of new drugs and regimens, such as new cancer targeting therapies, platinum and others, could improve the outcome. Capecitabine is a selective antitumor drug and thymidine phosphorylase (TP) is the crucial enzyme that converts the intermediate metabolite of capecitabine into active form 5-fluororacial in tumor tissues. Various studies in breast cancer models have indicated that accepting epirubicin cyclophosphamide/adriamycin cyclophosphamide (EC/AC) or containing taxol regimens can up-regulate the expression of TP in tumor tissues[24,25], and even just oral intake of cyclophosphamide also has similar effect[26]. Thus, cyclophosphamide, epirubicin and capecitabine (CEX) appeared to be a good partner for breast cancer treatment. Babyshkina et al[27] reported that TNBC patients receiving cyclophosphamide, adriamycin and capecitabine (CAX) regimen had a better prognosis in Russia. In contrast, other studies[28,29] analyzing capecitabine containing regimens did not observe increased

Table VIII. PTPN12 expression and its correlation with pCR prediction in TNBC and Luminal B subgroups.

	TNBC				Luminal B			
	N	No pCR	yes pCR	*p* value[a]	N	No pCR	yes pCR	*p* value[a]
Low	19	19 (100.0)	0 (0)	0.036	27	26 (96.3)	1 (3.7)	0.033
High	5	3 (60.0)	2 (40.0)		33	25 (75.8)	8 (24.2)	

[a]Fisher's Exact test

pCR rate. However, our work identified that CEX regimen is an independent risk factor for the pathological response. The clinical remission rate was statistically increased in CEX regimen group (71.4%). Similarly, pCR rate due to CEX regimen was 33.3% and was significantly higher in comparison to TEC and CEF group. It is important to mention here that low PTPN12 expression was an important factor to recommend CEX regimen for clinical remission. Thus, our study demonstrated an advantage of using capecitabine in neoadjuvant chemotherapy for breast cancer treatment and CEX regimen showed superior efficacy to TEC and CEF with the increased complete response rates pathologically and clinically.

Conclusions

We showed that cTNM classification was an independent risk factor correlated with PTPN12 expression. High PTPN12 expression appeared to be a protective factor for the clinical and pathological efficacy of NACT and seems helpful in planning NACT treatment. In addition, low PTPN12 expression played a role in identifying the sensitivity of tumors to CEX regimen, which was an independent factor for pCR. Even though we failed to confirm PTPN12 as an independent risk factor to predict the pathological efficacy of NACT for the entire cohort, high PTPN12 expression was a favorable factor to acquire pCR for the TNBC and luminal B subgroups. Furthermore, additional investigations would be required to determine the exact significance of PTPN12 expression for breast cancer prognosis after NACT.

Conflict of Interests

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