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Comprehensive analysis of the PTPN13 expression and its clinical implication in breast cancer

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Protein tyrosine phosphatases non-receptor 13 (PTPN13) could be a potential biomarker in breast cancer (BRCA), but its genetic variation and biological significance in BRCA remain undefined. Hereon, we comprehensively investigated the clinical implication of PTPN13 expression/gene mutation in BRCA. In our study, a total of 14 cases of triple-negative breast cancers (TNBC) treated with neoadjuvant therapy were enrolled, and post-operation TNBC tissues were collected for next-generation sequencing (NGS) analysis (422 genes including PTPN13). According to the disease-free survival (DFS) time, 14 TNBC patients were divided into Group A (long-DFS) and Group B (short-DFS). The NGS data displayed that the overall mutation rate of PTPN13 was 28.57% as the third highest mutated gene, and PTPN13 mutations appeared only in Group B with short-DFS. In addition, The Cancer Genome Atlas (TCGA) database demonstrated that PTPN13 was lower expressed in BRCA than in normal breast tissues. However, PTPN13 high expression was identified to be related to a favorable prognosis in BRCA using data from the Kaplan-Meier plotter. Moreover, Gene Set Enrichment Analysis (GSEA) revealed that PTPN13 is potentially involved in interferon signaling, JAK/STAT signaling, Wnt/β-catenin signaling, PTEN pathway, and MAPK6/MAPK4 signaling in BRCA. This study provided evidence that PTPN13 might be a tumor suppressor gene and a potential molecular target for BRCA, and genetic mutation and/or low expression of PTPN13 predicted an unfavorable prognosis in BRCA. The anticancer effect and molecular mechanism of PTPN13 in BRCA may be associated with some tumor-related signaling pathways.

Key words: PTPN13; BRCA; mutation; TCGA; prognosis

Breast cancer (BRCA) has surpassed lung cancer as the leading cause of cancer incidence worldwide in the Global Cancer Statistics 2020 report [1]. BRCA, as a heterogeneous disease [2], is primarily divided into three intrinsic molecular subtypes: hormone receptor-positive (HR+) luminal, human epidermal growth factor receptor 2 positive (HER2+), and triple-negative breast cancers (TNBC) [3,4]. However, BRCA is still incurable due to the various genome and/or transcriptome alterations [5]. Thus, novel and effective targeted therapies are urgently needed to improve the overall prognosis of BRCA.

Tyrosine phosphorylation, as a key post-translational modification, plays an important role in tumor cell proliferation, differentiation, and progression. The protein tyrosine phosphatases non-receptor 13 (PTPN13) is a class I non-receptor protein tyrosine phosphatase, which was the focus of our research [6]. PTPN13 was once called hPTP1E [6], PTP-BAS [7], and PTP-L1 [8] for being cloned by three different research teams, and then renamed FAS-associated phosphatase 1 (FAP-1) due to its interaction with Fas [9]. The PTPN13 gene is located on chromosome 4q21.3 and encodes the non-receptor protein phosphatase with the highest molecular weight (270 kDa; 2,466 amino acids) [10]. PTPN13 is a soluble and cytosolic non-receptor protein that can be translocated to the sub-membranous and nuclear compartments during mitosis [11]. An early study showed that high PTPN13 expression is associated with better prognosis in solid tumors, including BRCA [12]. Instead, recent studies indicated a reverse value of PTPN13 in FAS-induced apoptosis and suggested a pro-tumor value in hematopoietic cancers [13]. The PTPN13 phosphatase may play a dual role in cancers. Until now, the clinical function and molecular

mechanism of PTPN13 in BRCA remain largely unidentified [14]. In addition, PTPN13 variation and its consequences in BRCA have not yet been reported [15]. Therefore, given the higher tumor mutational burden (TMB) of TNBC [16], we retrospectively enrolled 14 locally advanced TNBC patients with residual cancer burden (RCB) tissues after neoadjuvant chemotherapy (NAC). The postoperative BRCA tissues were collected for next-generation sequencing (NGS) analysis (422 common tumor genes including PTPN13) to explore gene mutation of PTPN13 and tumor heterogeneity in TNBC [17]. It is remarkable that gene variation of PTPN13, as the third highest mutated gene, appeared only in patients with short disease-free survival (DFS ≤12 months). However, the multiple biological mechanisms of PTPN13 in BRCA need to be comprehensively researched.

For this study, we comprehensively investigated the clinical implication of PTPN13 expression/gene mutation in BRCA and revealed new insights into the molecular mechanisms of its anticancer effect. PTPN13 is expected to become a candidate biomarker and a potential target for BRCA clinical management.

Patients and methods

Patients and specimens. A total of 14 adult female BRCA patients were enrolled in this study, who were diagnosed with stage IIb–IIIc invasive TNBC and received 4–8 cycles of NAC with paclitaxel and anthracycline at the Breast Center of the Fourth Hospital of Hebei Medical University from January 2014 to December 2016. The pathological examination after radical surgery demonstrated that all cases were non-pathologic complete response (non-pCR) TNBC with residual tumor cells in the surgical breast specimen or draining nodes. The post-operational formalin-fixed paraffin-embedded (FFPE) samples from the 14 TNBC patients above were collected for targeted NGS analysis in the Nanjing Shihe Geneseeq Biotechnology Inc. (Nanjing, China). All 14 FFPE tissues were considered qualified with at least more than 20% tumor cell content. All patients have been followed up by physical examination and/or imaging studies after the operation until July 2022. The primary endpoint was disease-free survival (DFS). Our study was conducted in accordance with the 2013 (7th Edition) Declaration of Helsinki and granted institutional ethics committee of the Fourth Hospital of Hebei Medical University (No. 2019MEC065) with written informed consent from all patients.

DNA extraction and targeted NGS. About 6–8 sections (10 μm thickness) from every FFPE block were deparaffinized with xylene, then tumor genomic DNA extraction was performed with the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendation. The extracted genomic DNA was cleaved into fragments (300–350 bp) by Diagenode Bioruptor Plus (Bioruptor, Belgium). Subsequently, a custom NGS panel was provided for hybridization enrichment, targeting the whole exons and partial introns of 422 cancer-related genes (Supplementary Figure S1). DNA libraries were subjected to hybridization using Dynabeads M-270 (Thermo Fisher). FFPE samples were sequenced with a median sequencing depth of 600× on Illumina Hiseq 4000 platform (Illumina, San Diego, CA, USA). The results of sequencing data underwent the FASTQ file quality control (QC) using Trimmomatic [18] and then qualified reads were mapped to the reference human genome by Burrows-Wheeler Aligner (BWA-MEM, v.0.7.12) [19]. The Genome Analysis Toolkit (GATK, version 3.4-0) was modified and used to locally realign the BAMs files at intervals with indel mismatches and recalibrate base quality scores of reads in BAM files. The routine result report contained a listing of identified gene alterations.

PTPN13 expression in BRCA. The transcription levels of PTPN13 in 33 different cancers were analyzed using RNA-sequencing (RNA-seq) expression data in The Cancer Genome Atlas (TCGA) (https://cancergenome.nil.gov). Different cancer types (tumor/normal) were plotted on the x-axis and PTPN13 expression was plotted on the y-axis, and the gene expression level was presented as log2 (TPM+1) in Figure 2. It contained the RNA-seq data from 1,099 BRCA specimens and 292 normal breast tissues. The RNA-seq information and corresponding clinical data were obtained using the Data Transfer Tool (Workflow Type: HTSeq-TPM). Other clinical and pathological characteristics included in our analysis were as follows: ER, PR, HER2, molecular subtype, pathology stage, and histological type. The Human Protein Atlas (HPA) (www.proteinatlas.org) covers virtually all human protein-coding genes in cells, tissues, and organs [20]. This study used the HPA database to assess the PTPN13 protein profile in BRCA tumors and normal tissues. The representative immunohistochemistry (IHC) images of PTPN13 with different expression levels are shown in Figure 3.

Prognostic analysis of PTPN13. The online Kaplan-Meier plotter (https://kmplot.com/analysis accessed on 25 July 2022) is a meta-analysis-based database to assess survival data of approximately 54,000 genes (mRNA, miRNA, protein) in 21 cancer types, including breast, ovarian, lung, and gastric cancer [21]. Our study evaluated the prognostic significance of PTPN13 expression in BRCA. According to the median expression level of PTPN13, the cohort was divided into the high-PTPN13 and the low-PTPN13 expression group. Overall survival (OS) and relapse-free survival (RFS) were the primary endpoints of our analysis. Hazard ratios (HRs) with a 95% confidence interval (CI) were computed for survival analysis, and p-values were calculated using the log-rank test.

Genomic analysis of PTPN13. The cBio Cancer Genomics Portal (cBio-Portal) (https://www.cbioportal.org) is an open resource for the interactive exploration of numerous multidimensional cancer genomics datasets, which contains more than 5,000 tumor samples from 147 cancer studies currently [22]. Our study mainly evaluates the somatic gene mutation/

variation and copy number variation (CNV) profile of PTPN13 in BRCA in the cBioPortal database.

Function enrichment analysis of PTPN13 in BRCA. The R package DESeq2 was used to perform the differentially expressed gene (DEG) analysis between the high and low PTPN13 mRNA expression groups [23]. Adjusted p-value <0.05 and |log2 Fold Change (FC)| >2 were set as the threshold of the DEGs. Gene set enrichment analysis (GSEA) was carried out using the R package software (v4.0.3) [24]. Related pathways with adjusted p<0.05, false discovery rate (FDR) ≤0.25, and normalized enrichment score (|NES|) >1 were regarded as statistically significantly enriched terms of PTPN13 in BRCA.

Statistical analysis. A descriptive analysis of BRCA patients was conducted for clinicopathological features. Data are shown as mean ± standard deviation (SD). SPSS software (version 25.0) and R (version 4.0.5) were used for statistical analyses. The χ^2 and Fisher tests were utilized to analyze the clinical factors in different DFS groups. Wilcoxon rank-sum test and paired sample t-test were used to assess the statistical significance of PTPN13 expression between the two groups. All of the tests were two-sided, and p<0.05 was considered statistically significant.

Results

Genetic mutation in TNBC patients identified by NGS. The clinicopathological features of 14 TNBC patients are listed in Table 1. According to the DFS time, 7 patients were assigned to Group A: long-DFS >12 months, while the other 7 cases were enrolled into Group B: short-DFS ≤12 months. The median age of the 14 TNBC patients was 54 years. There was no significant difference in age, menopausal, performance status, tumor size, lymph node metastasis, TNM stage, and Ki-67 score between the two groups. Subsequently, 422 cancer-related genes of the customized NGS panel (Supplementary Figure S1) were screened per post-operational TNBC tissue. Figure 1A represented the overall genetic alteration map and gene mutation number of each case. There were 72 points of gene alteration detected in the 14 patients with a median mutation number of 5. The genetic alteration was classified into 6 types, with the majority of a missense mutation in 50.0% (36/72) patients (Figure 1B). It demonstrated that both the variation type and mutation frequency of Group B were higher than those of Group A. Figures 1C and 1D showed the mutation profile of the two groups separately, with a higher missense frequency of 57.0% (24/42) in Group B compared with 40.0% rate (12/30) in the Group A. Among the forefront 30 mutation genes detected by this 422-gene NGS panel, TP53 (85.7%, 12/14), RB1 (42.9%, 6/14), and PTPN13 (28.6%, 4/14) were the top 3 genes with the highest mutation rate (Figure 1A). It is remarkable that the genetic variation of PTPN13 appeared only in Group B: short-DFS patients with 2 missense and 2 frameshift mutations. Moreover, PTPN13 mutation demonstrated a significant difference between the two groups (p=0.035; Table 1).

Table 1. Baseline characteristics of patients (n=14).

Characteristics	N (n=14)	Group A: long DFS (n=7), n (%)	Group B: short DFS (n=7), n (%)	p-value
Age (years)				0.500
<50	7	3 (42.86)	4 (57.14)	
≥50	7	4 (57.14)	3 (42.86)	
Menopausal				0.500
Premenopausal	7	3 (42.86)	4 (57.14)	
Postmenopausal	7	4 (57.14)	3 (42.86)	
ECOG performance status				0.769
0	12	6 (85.71)	6 (85.71)	
1	2	1 (14.29)	1 (14.29)	
Tumor size				1.000
T1	1	0 (14.0)	1 (14.29)	
T2	10	5 (71.43)	5 (71.43)	
T3	1	1 (14.29)	0 (20.5)	
T4	2	1 (14.29)	1 (14.3)	
Lymph node status				0.493
N0	1	1 (14.29)	0 (0.00)	
N1	3	1 (14.29)	2 (28.57)	
N2	4	1 (14.29)	3 (42.86)	
N3	6	4 (57.14)	2 (28.57)	
TNM stage				1.000
IIb	3	1 (14.29)	2 (28.57)	
IIIa	2	1 (14.29)	1 (14.29)	
IIIb	1	1 (14.29)	0 (0.00)	
IIIc	8	4 (57.14)	4 (57.14)	
Ki-67 status				0.769
<20%	2	1 (14.29)	1 (14.29)	
≥20%	12	6 (85.71)	6 (85.71)	
PTPN13 gene of NGS				0.035
Mutation type	4	0	4	
Wide type	10	7	3	

Abbreviations: DFS-disease-free survival; NGS-next generation sequencing

Low PTPN13 mRNA expression in BRCA. We searched PTPN13 mRNA expressions in 33 different types of tumors from TCGA database (Figure 2A). The results reflected that PTPN13 mRNA was lower expressed in 18 tumor types. PTPN13 expression was significantly lower in the BRCA compared to the normal group in both total specimens (3.037±1.625 vs. 3.316±0.775; p<0.001; Figure 2B) and paired cases (4.266±1.373 vs. 4.641±0.650; p<0.001; Figure 2C). Besides, PTPN13 expression was significantly associated with ER/PR positive status (p<0.001, Figures 2D, 2E), but no significant correlation with HER2 status was found (p=0.053, Figure 2F). In addition, PTPN13 expression was strongly related to molecular subtypes of BRCA with the highest PTPN13 expression in the luminal A subtype (p<0.001, Figure 2G). However, PTPN13 mRNA expression was not associated with the TNM stage of BRCA (p=0.693,

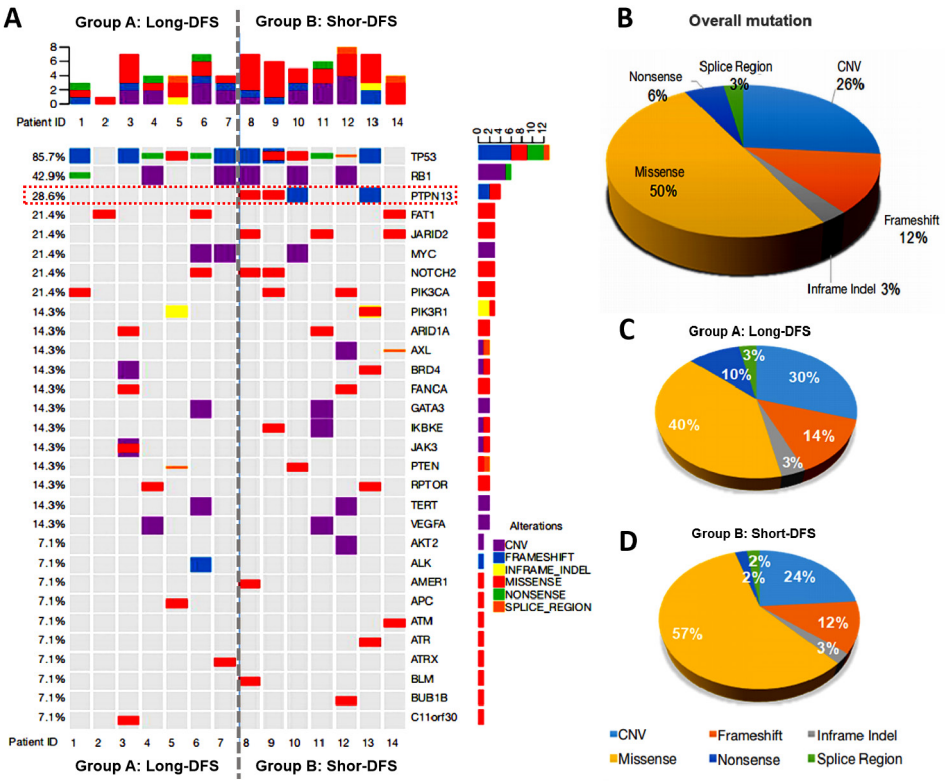

Figure 1. Gene mutation map of non-pCR TNBC tissues analyzed by NGS. A) The overall profile of genetic alteration and mutation types in 14 patients. Patients were separated into two groups, Group A: long-DFS and Group B: short-DFS. The mutation percentage of each gene is displayed on the left. The gene names and mutation types were shown on the right. B) The distribution of mutation numbers and types in overall patients. Mutation numbers were classified according to the 6 mutation types. C) Mutation distribution in Group A: long-DFS lesion. Mutation distribution in Group B: short-DFS lesion. Abbreviations: TNBC-triple-negative breast cancer; DFS-disease-free survival; TNBC- triple-negative breast cancer; NGS-next generation sequencing; CNV-copy number variation

Figure 2H) but it was significantly higher-expressed in the invasive lobular carcinoma (ILC) group than in the invasive ductal carcinoma (IDC) group (p=0.034; Figure 2I).

PTPN13 expression in BRCA by immunohistochemistry. In the HPA database, we assessed the expression pattern of the PTPN13 protein by IHC staining with the specific mouse antibody (CAB002213, Leica Biosystems, Germany). The results demonstrated that PTPN13 exhibited a subcellular location in the cytoplasm or membrane of BRCA tissue (Figure 3A) and normal lymph node tissues (Figure 3B). The representative IHC images of PTPN13 with high-expression, low-expression, and moderate-expression are shown in Figures 3A, 3C, and 3D, respectively.

Prognostic implication of PTPN13 in BRCA. In the Kaplan-Meier plotter database, we validated the correlation between PTPN13 expression and the prognosis of BRCA patients. The results implied that PTPN13 expression was not significantly associated with OS of BRCA patients (HR=0.71, 95% CI: 0.71–1.03, p=0.11; Figure 4A). However, according to the subgroup analyses, we found that high PTPN13 expression demonstrated a better OS in HER2- BRCA patients (HR=0.8, 95% CI: 0.64–1.00, p=0.049; Figure 4B). On the contrary, there was no statistical difference in the HER2+ BRCA group (HR=1.11, 95% CI: 0.77–1.60, p=0.56; Figure 4C). Besides, high PTPN13 expression demonstrated a similar favorable prognosis of RFS in the total population (HR=0.83, 95% CI: 0.75–0.92, p<0.001; Figure 4D) and HER2– BRCA group (HR=0.79, 95% CI: 0.7–0.88, p<0.001; Figure 4E). But PTPN13 expression was not associated with RFS in the HER2+BRCA group (HR=0.92, 95% CI: 0.74–1.15, p=0.47; Figure 4F).

Genomic alterations pattern of PTPN13 in BRCA. The overall analysis demonstrated a relatively lower mutation rate of the PTPN13 gene in BRCA tissues among the pan-cancer data in the cBio-Portal database (Figure 5A). The average gene alteration frequency of PTPN13 was 1.8% in the 12 representative BRCA studies (Figure 5B), including missense mutation, missense splice mutation, truncating mutation, amplification (AMP), and deep deletion (Figure 5C). It was remarkable that the highest alteration frequency of PTPN13 was 11.35% (43/379 cases) in the study of The Metastatic Breast Cancer Project (Provisional, December 2021) with an AMP rate of 6.07% (23/379 cases) and deep deletion rate of 3.69% (14/379 cases) (Figure 5B). AMP seemed to be the

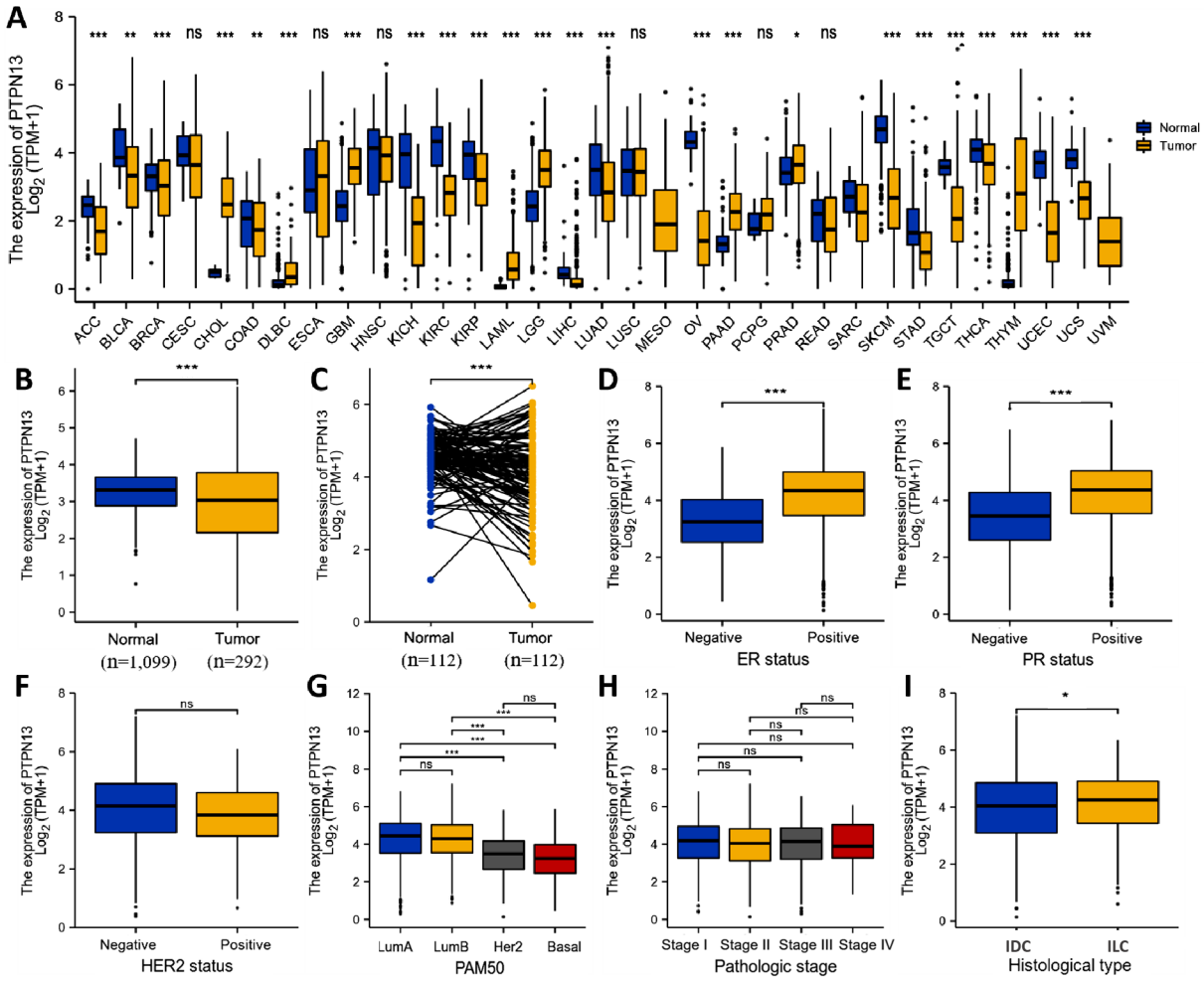

Figure 2. PTPN13 expression in BRCA and other types of human cancer from TCGA data. A) PTPN13 expression levels in different tumor types from TCGA database. B) Expression levels of PTPN13 mRNA in BRCA (n=1,099) and normal tissue (n=292); C) The expression of PTPN13 in BRCA (n=112) and its paired adjacent tissues (n=112). D) The association of PTPN13 expression and ER status in BRCA. E) The association of PTPN13 expression and PR status in BRCA. F) The association of PTPN13 expression and HER2 status in BRCA. G) The association of PTPN13 expression and different subtypes in BRCA. H) The association of PTPN13 expression and pathologic stages in BRCA. I) The association of PTPN13 expression and histological types in BRCA. *p<0.05, **p<0.01, ***p<0.001. Abbreviations: PTPN13-protein tyrosine phosphatases non-receptor 13; BRCA-breast cancer; TCGA-The Cancer Genome Atlas; ER-estrogen-receptor; PR-progesterone receptor; HER2-human epidermal growth factor receptor 2; IDC-invasive ductal carcinoma; ILC-invasive lobular carcinoma

most common type of PTPN13 gene variation in BRCA (Figure 5C). Subsequently, we analyzed the association between PTPN13 mRNA expression and its genome status to further verify the regulation mechanism of PTPN13 expression. It showed that AMP led to a relatively higher expression of PTPN13, whereas deep deletion related to a lower PTPN13 mRNA expression (Figure 5D). However, CNV of PTPN13 made no significant difference to the prognosis of BRCA patients (log-rank p=0.285; Figure 5E).

Functional enrichment analysis of PTPN13 in BRCA. In TCGA BRCA database, we enriched the functional pathways to visualize the connection between PTPN13 and its DEGs. The PTPN13-associated DEGs were demonstrated in the volcano map (Figure 6A). We identified a total of candidate 877 DEGs with |log2 (FC)| >2 and adjusted p-value (p. adj) <0.05, 685 upregulated genes and 192 downregulated genes. The top 5 involved functions of these DEGs covered UV response, reactive oxygen species pathway, IL6-JAK-STAT3 signaling, α-interferon response, and spermatogenesis (Figure 6B). The network of protein-protein interactions (PPIs) containing top significant DEGs was shown in Figure 6C. In addition, the enriched tumor-related pathways of GSEA analyses were involved in interferon signaling (p. adj=0.030, Figure 6D), JAK/STAT signaling p. adj=0.030, Figure 6E), Wnt/β-catenin signaling (p. adj=0.030, Figure 6F), regulation of PTEN stability and activity (p. adj=0.042, Figure 6G), and the MAPK6/MAPK4 signaling pathway (p. adj=0.042, Figure 6H). However, WP apoptosis modulation

and signaling were not significantly enriched in this GSEA analysis (p. adj=0.059, Figure 6I).

Discussion

Genetic alteration in oncogenes and/or anti-oncogenes plays a prominent role in tumor development, so NGS analysis came into being as an effective technology for understanding the underlying heterogeneity in tumors [25]. Our study involved 14 non-PCR TNBC patients and sequenced postoperative tumor tissues by NGS technology. The top 2 genes with the highest mutation frequency were TP53 and RB1 as critical tumor suppressors [26], which were detected in both Group A and Group B. It was remarkable that PTPN13, as the third highest mutated gene, appeared only in the short-DFS patients. PTPN13, as a class I non-receptor protein tyrosine phosphatase, is a soluble protein that could be transported to the nuclear compartments and sub-membranous during cell mitosis [11]. Our study also demonstrated that PTPN13 exhibited a subcellular location in the cytoplasm or membrane of BRCA tissue in the HPA database. In addition, we found that PTPN13 was downregulated in BRCA compared with adjacent normal tissues, but PTPN13 expression was relatively higher in HR+ and ILC patients, which displayed that PTPN13 expression may be linked to less aggressive BRCA tumors. Meanwhile, another study observed an obvious decrease in PTPN13 expression level from normal breast tissues to metastatic BRCA tissues by IHC [27]. Moreover, PTPN13 high expression predicted favorable OS and RFS in HER2– BRCA patients in our analysis. Besides, an early study also implied that high PTPN13 expression was significantly related to a better prognosis (p=0.01 and RR=0.48 in multivariate analysis) [12]. Above all, PTPN13 seemed to be a potential biomarker of favorable prognosis in solid BRCA tumors. However, molecular mechanisms underlying the anticancer effect of PTPN13 in BRCA remain unclear and need more specific research [14].

In our data, we mainly enriched the cancer-related signaling pathways of PTPN13-associated DEGs in BRCA, such as interferon signaling, JAK/STAT signaling, Wnt/β-catenin signaling, PTEN pathway, and MAPK6/MAPK4 signaling. Previous studies have shown that PTPN13 maybe inhibited tumor cell invasion and aggressiveness through the regulation of these cancer-related genes. SRC, as a pro-oncogenic tyrosine kinase, was negatively regulated by PTPN13 in BRCA [27]. In addition, there may be a HER2-induced feedback mechanism that induced PTPN13 expression and thereby PTPN13 negatively regulated HER2 signaling pathways [28]. Meanwhile, there was a similar tendency that PTPN13 was higher-expressed in HER2 negative group in our data. In addition, Vermeer et al. revealed a novel signaling pathway regulated by PTPN13 that involved ephrinB1, HER2, and ERK in BRCA cell lines. They discovered that PTPN13 silencing by shRNA led to HER2 overexpression, ERK1/2 phosphorylation, and MAPK pathway activation in MDA-MB-468 breast cancer cells [29]. Moreover, PTPN13 was also involved in the Wnt/β-catenin signaling by negatively regulating its substrate thyroid hormone receptor interactor 6 (TRIP6) which could promote cell mobility [30]. Another study demonstrated that PTPN13 might hinder epithelial-mesenchymal transition (EMT) with its positive role in desmosome formation and cell junction stabilization *in vitro* study of MDA-MB-231 cells and *in vivo*

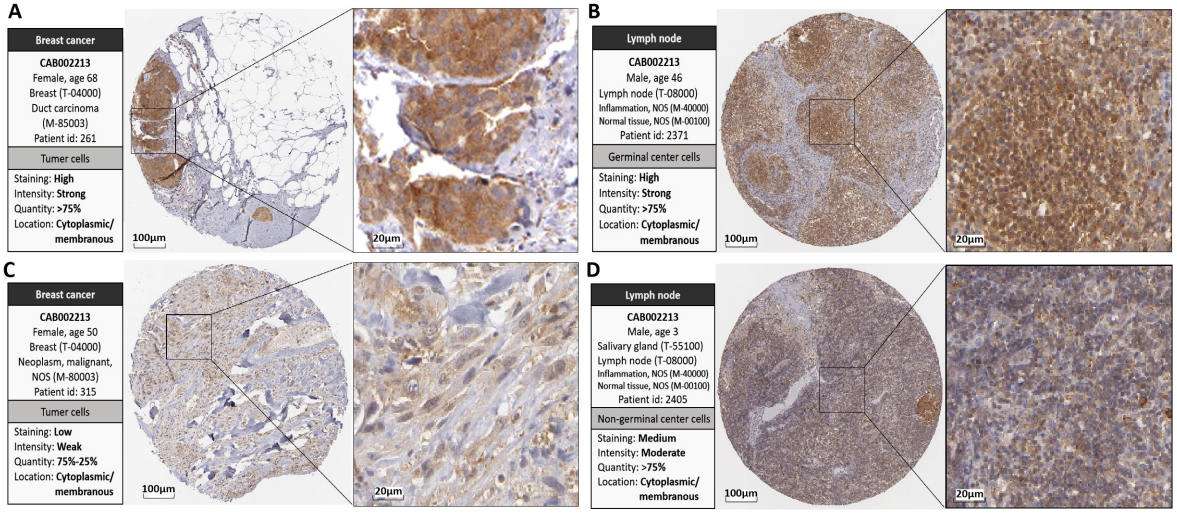

Figure 3. PTPN13 protein expression in cancerous breast tissues and nontumorous lymph node tissues based on IHC data from the HPA database (antibody: CAB002213). A) Representative high IHC staining patterns of PTPN13 in BRCA tissues. B) Representative high IHC staining patterns of PTPN13 in lymph node tissues. C) Representative low IHC staining patterns of PTPN13 in BRCA tissues. D) Representative medium IHC staining patterns of PTPN13 in lymph node tissues. Abbreviations: PTPN13-protein tyrosine phosphatases non-receptor 13; IHC-immunohistochemistry; BRCA-breast cancer

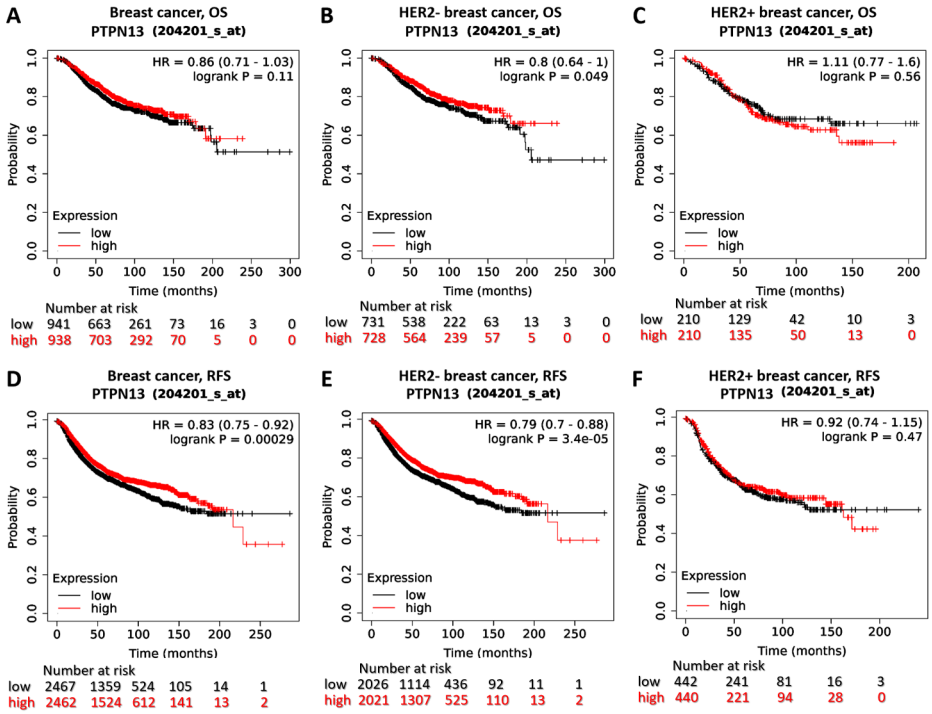

Figure 4. The prognostic value of PTPN13 expression in BRCA from the Kaplan-Meier plotter database. A) OS survival curves of breast cancer. B) OS survival curves of HER2– breast cancer. C) OS survival curves of HER2+ breast cancer. D) RFS survival curves of breast cancer. (E) RFS survival curves of HER2– breast cancer. F) RFS survival curves of HER2+ breast cancer. Abbreviations: PTPN13-protein tyrosine phosphatases non-receptor 13; BRCA-breast cancer; OS-overall survival; RFS-relapse-free survival; HER2-human epidermal growth factor receptor 2

model of transgenic BRCA mice [31]. Meanwhile, Fan et al. illustrated that PTPN13 could dephosphorylate junctional adhesion molecule-A (JAM-A) to maintain the stabilization of the transmembrane component [32]. In the above, PTPN13 had a positive effect on interacting with tight junctions and cytoskeleton molecules in epithelial cells junctions [33].

Besides, the PI3K/PTEN pathway was often overactivated in BRCA, and Bompard et al. demonstrated that PTPN13 could inhibit PI3K function in MCF7 breast cancer cells [34]. Meanwhile, yeast two-hybrid and GST pull-down assays revealed that the second PDZ domain of PTPN13 could bind to phosphatase and tensin homolog (PTEN) for regulating the PI3K signaling pathway [35]. Our data also enriched the PTEN pathway in the GSEA analysis. Moreover, PTPN13 was upregulated by antiestrogen agents, which in turn promoted tumor cells' apoptosis [36]. However, apoptosis pathways were not significantly enriched in our data. The first reported evidence of FAS-mediated apoptosis was the association of PTPN13 with the FAS death receptor [9]. In hematopoietic cancers, some mechanistic studies indicated PTPN13 had a negative role in FAS-induced apoptosis and thereby promoted tumor progression [13]. Nevertheless, the accurate roles of PTPN13 in solid tumors were unclear owing to multiple signaling pathways, particularly in terms of cell junction maintenance. Given the resistance to FAS-mediated apoptosis of PTPN13, some scholars have begun to study the relationship between PTPN13 expression and the resistance to cancer treatment in BRCA. It was reported that dephosphorylated ephrin B1, directly regulated by PTPN13, could bind to mitotic spindle microtubules and then increase the sensitivity to paclitaxel in breast cancer cell lines. In short, BRCA tumors with low PTPN13 expression would lead to resistance to paclitaxel [37]In addition to the above, PTPN13 gene expression and biological activities could also be regulated by some signaling pathways. It was revealed that STAT3, HDAC5 [38], and SMYD2 [39] were involved in the transcriptional regulation of PTPN13 by chromatin immunoprecipitation assay. It was demonstrated that the pro-tumor transcription factor STAT3 in combination with the nuclear co-repressor HDAC5, inhibited PTPN13 transcription in squamous cell lung carcinoma cell lines (HCC-1588 and SK-MES-1) after stimulation by the pro-tumor interleukin 6 [38]. The transcription factor SMYD2 was a negative regulator of PTPN13 transcription, which promotes tumorigenesis in mice with xenografts of TNBC cell lines (MDA-MB231 and MDA-MB468) [39].

In terms of PTPN13 genetic alterations, the overall gene mutation rate of PTPN13 was not prominent in BRCA, and variation mechanism research remained less specific at present. There are currently four isoforms of PTPN13 [40],

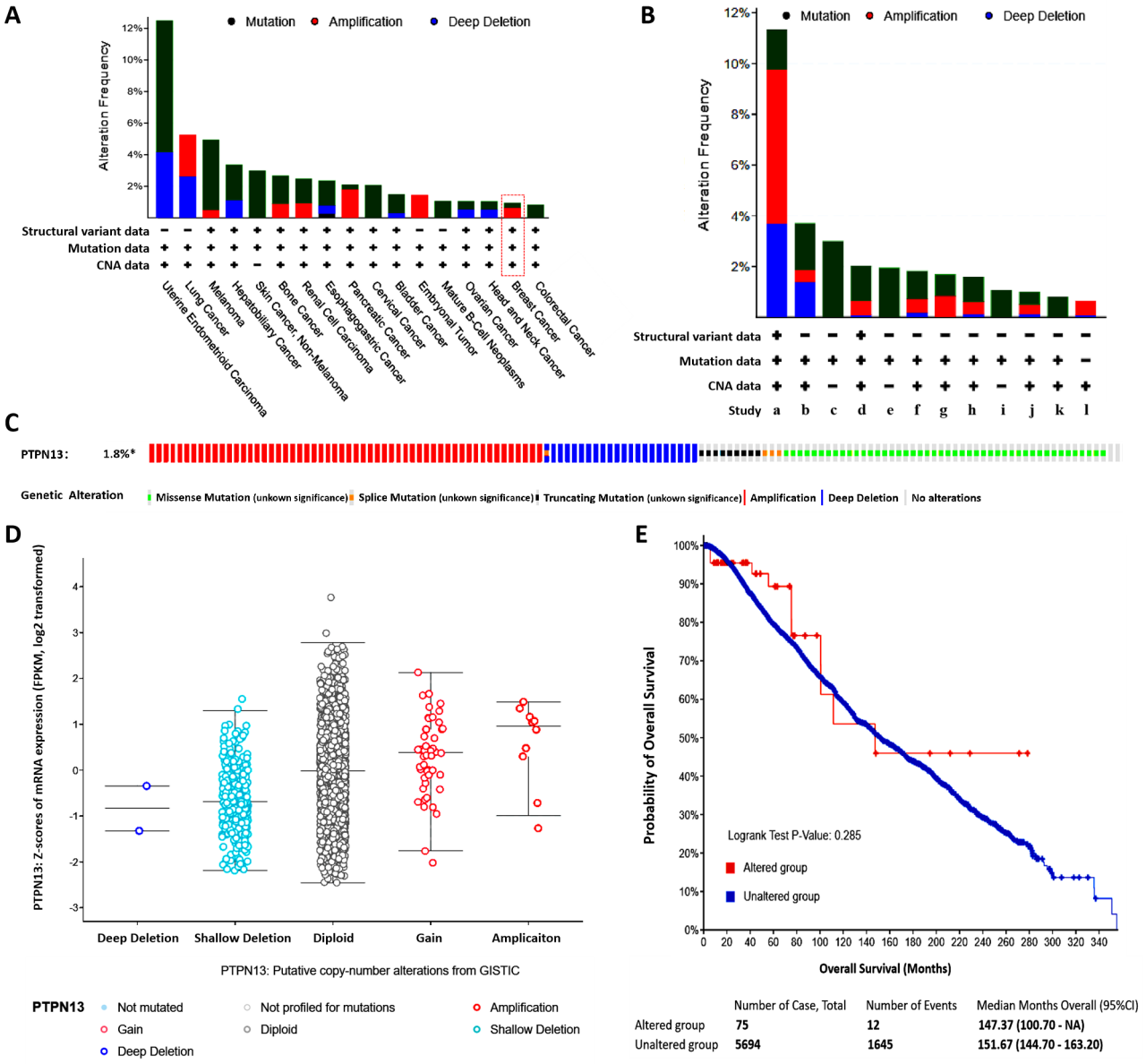

Figure 5. PTPN13 genomic alterations analysis in BRCA. A) Landscape of PTPN13 genetic alterations in pan-cancer. (B) Genetic alterations of PTPN13 in BRCA from 12 study data. Study (a) The Metastatic Breast Cancer Project (Provisional, December 2021); (b) Metastatic Breast Cancer (INSERM, PLoS Med 2016); (c) Breast Invasive Carcinoma (Sanger, Nature 2012); (d) Breast Invasive Carcinoma (TCGA, PanCancer Atlas); (e) Breast Invasive Carcinoma (Broad, Nature 2012); (f) Breast Invasive Carcinoma (TCGA, Firehose Legacy); (g) The Metastatic Breast Cancer Project (Archived, 2020); (h) Breast Invasive Carcinoma (TCGA, Cell 2015); (i) Breast Cancer (SMC 2018); (j) Breast Invasive Carcinoma (TCGA, Nature 2012); (k) Proteogenomic landscape of breast cancer (CPTAC, Cell 2020); (l) Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016). C) The overall genomic alterations of PTPN13 expression in BRCA. D) PTPN13 expression in different alternation groups. E) The prognosis of PTPN13 based on its alternation condition. Abbreviations: PTPN13-protein tyrosine phosphatases non-receptor 13; BRCA-breast cancer; TCGA-The Cancer Genome Atlas

but the regulations and consequences of PTPN13 alternative splicing remain largely unexplored. To date, only one published NGS research found a decrease of over 25% in PTPN13 exon inclusion rate as the consequence of hypoxia in prostate adenocarcinoma cells, but the functions of its spliced isoforms remain unknown [41]. Besides, PTPN13 gene deletion has been observed in 37% of non-small cell lung cancer (NSCLC) [42], with higher mutation prevalence in metastatic lung cancer [43]. PTPN13 genetic variations were worthy of further exploration and research. However, only a limited number of studies reported that gene mutations of PTPN13 could attenuate its anticancer activity, leading to an unfavorable prognosis in lung cancer [44] and gastric cancer [45]. In our study, we found that PTPN13 genetic variation

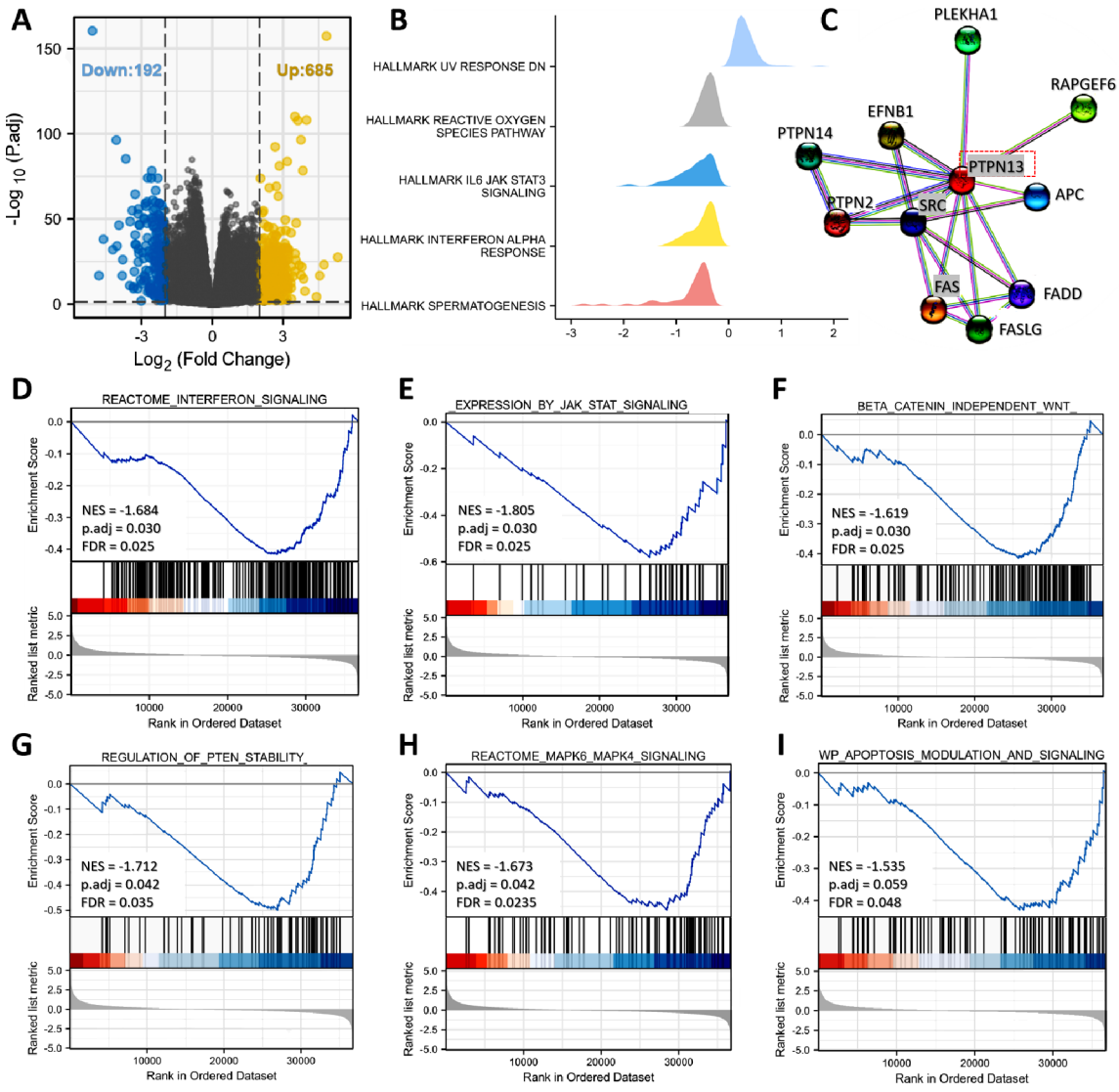

Figure 6. Function and pathway enrichment analyses of PTPN13 in BRCA. A) Volcano Plot of PTPN13 associated differentially expressed genes (DEGs) in TCGA BRCA database. B) The top 5 associated pathways in gene set enrichment analysis (GSEA). C) The protein-protein interaction (PPI) network of the top PTPN13 DEGs. D–I) GSEA functional enrichment of PTPN13 associated DEGs in BRCA. (D) REACTOME interferon signaling. (E) REACTOME gene and protein expression by JAK-STAT signaling pathway. (F) REACTOME Wnt/β-catenin signaling. (G) REACTOME regulation of PTEN stability and activity. (H) REACTOME MAPK6/MAPK4 signaling. (I) WP apoptosis modulation and signaling. Abbreviations: PTPN13-protein tyrosine phosphatases non-receptor 13; BRCA-breast cancer

appeared only in short-DFS patients, implying that PTPN13 gene mutation similarly led to a poor outcome in BRCA. However, further research is needed in this field of PTPN13 genetic variation and its biological implication on BRCA.

For this research, we explored the expression, prognosis, genome variation, and enrichment analysis of PTPN13 in a variety of BRCA databases. It was demonstrated that PTPN13, as a favorable prognostic biomarker, was relatively lower-expressed in BRCA. And genetic mutation of PTPN13 may have a negative effect on its anticancer activities. Although these results improved our understanding of the clinical significance of PTPN13 in BRCA, there were still several limitations in our study. First, the main deficiency was that these findings were based on limited retrospective studies with a small sample size, which should be confirmed in large-scale prospective cohort studies in the future. Second, more comprehensive research is necessary to identify the genomic alterations in the DNA, mRNA, and protein levels of PTPN13 in our BRCA tissues and adjacent normal breast tissues. Third, the accurate biological significance of PTPN13 in BRCA is necessary to be validated in cultured cells *in vitro* and *in vivo* in animal models. Furthermore, we may have

missed additional relevant signaling pathways due to statistical reasons, and these enriched signaling pathways of tumor progression should be verified in a wide array of research.

In summary, our research demonstrated the clinical implications of PTPN13 in BRCA. It provided evidence that a relatively low expression and/or gene variation of PTPN13 were related to unfavorable outcomes of BRCA patients. In addition, functional enrichment analysis of PTPN13 provided important clues for its underlying biological mechanisms in BRCA tumor progression. This study that revealed PTPN13 may be a novel biomarker of BRCA prognosis and a potential candidate target for BRCA treatment, expecting to provide valuable information for clinical diagnosis and targeted therapy for BRCA.

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Supplementary information is available in the online version of the paper.

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