

PTPRA facilitates cancer growth and migration via the TNF- α -mediated PTPRA-NF- κ B pathway in MCF-7 breast cancer cells

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Abstract. Protein tyrosine phosphatase receptor type A (PTPRA), one of the classic protein tyrosine phosphatases, is crucial for modulating tumorigenesis and metastasis in breast cancer; however, its functional mechanism has not fully elucidated. The present study assessed PTPRA expression and estimated its clinical impact on survival using the Gene Expression Profiling Interactive Analysis database (GEPIA). Growth curves, colony formations and Transwell assays were utilized to examine cell proliferation and migration. Additionally, luciferase reporter assays were used to examine the potential tumor signaling pathways targeted by PTPRA in HEK293T cells. Furthermore, quantitative PCR (qPCR) was utilized to confirm the transcriptional regulation of PTPRA expression. Bioinformatic analyses of data from GEPIA identified PTPRA overexpression in patients with breast cancer. The growth curve, colony formation and transwell experiments demonstrated that PTPRA upregulation significantly promoted the cell proliferation and migration of MCF-7 breast cancer cells. In contrast, PTPRA knockdown significantly attenuated cell proliferation and migration. Mechanistic experiments revealed that the transcriptional activity of NF- κ B was higher compared with other classic tumor pathways when they were activated by PTPRA in HEK293T cells. Furthermore, the transcriptional activity of NF- κ B was altered in a PTPRA-dose-dependent manner. Additionally, following exposure to TNF- α , PTPRA-deficient MCF-7 cells exhibited lower NF- κ B transcriptional activity compared with normal

control cells. The results of the present study demonstrate that PTPRA overexpression accelerates inflammatory tumor phenotypes in breast cancer and that the TNF- α -mediated PTPRA-NF- κ B pathway may offer novel insight into early diagnosis and optimum treatment for breast cancer.

Introduction

Breast carcinoma is a primary cause of cancer-associated mortality in women aged 20-59 years. Statistical studies have demonstrated that the incidence of breast carcinoma is increasing annually and accounts for 30% of new cancer diagnoses in women alone in USA in 2019 (1). The therapeutic modalities that are currently applied are selected primarily according to the most extensively studied biomarkers: Estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (2). However, previous studies have demonstrated that the occurrence, tumorigenesis and metastasis of breast cancer are controlled by complex signaling networks (3-5). Thus, a complete understanding of the molecular mechanism of breast carcinogenesis is required to eliminate obstacles in the early detection and treatment of breast cancer.

Aberrant protein phosphorylation is one of most typical characteristics of tumor cells. Protein tyrosine phosphatases (PTPs) are critical enzymes that modulate the phosphorylation status of intracellular signaling molecules (6-8). It is well-established that PTPs negatively or positively regulate cancer-associated signaling pathways in breast cancer (8-10). PTP1B overexpression promotes proliferation and migration by regulating the phosphorylation of steroid receptor coactivator (11). PTP δ has been predicted to be an enhancer of tumorigenicity and its high expression has been tested in clinical breast cancer samples (12). Furthermore, PTP receptor type (PTPR) K potentially serves a negative role in breast cancer and a low PTPRK transcript level is associated with poor prognosis and low survival rates (13). Furthermore, tumor function inhibition via PTPN12 expression alteration suppresses breast cancer development and metastasis *in vivo* (14). Additionally, treatment of MCF-7 cells with c-Jun N-terminal kinase or extracellular signal-regulated kinase inhibitors partially rescue the effects of PTPRM knockdown

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on cell migration, indicating that PTPRM inhibits tumor metastasis by decreasing the activity of oncogenic protein tyrosine kinases (13,15).

Similar to other PTPs, PTPRA is closely associated with the tumorigenic phenotype of breast cancer via its control of the balance between PTKs and PTPs (16). A significant increase in PTPRA the transcription and translation levels has been confirmed in the majority of primary breast cancer types (16-18). Nonetheless, the role of PTPRA in breast cancer remains controversial. Ardini *et al* demonstrated that PTPA is an inhibitor of breast cancer cell proliferation and significantly delays cancer cell migration and invasion *in vivo* and *in vitro* (10), while other *in vivo* studies indicate that PTPRA enhances malignant activities, such as migration and invasion of tumor cells (16,17).

Mechanistically, PTPs, including PTPRA, are primarily physiological upstream activators of oncogenic SRC that act by dephosphorylating key signaling factors (19). Certain PTPs also directly interact with cell adhesion molecules, such as E-cadherin and β -catenin, to regulate cancer cell transformation (6). Furthermore, PTPRA has been reported to respond to different stimuli, such as insulin-like growth factor (IGF)-1, and activate IGF-1-mediated downstream signaling pathways that are critical in tumorigenesis and metastasis (20). Therefore, the present study concluded that further work concerning the precise molecular and cellular mechanisms is still essential to elucidate the role of PTPRA in breast cancer.

The present study clarified the oncogenic role of PTPRA and its underlying mechanism in breast cancer using loss and gain of function analyses, demonstrating the effect of PTPRA on the proliferation, colony formation and migration of MCF-7 cells. Furthermore, a luciferase reporter gene assay was used to screen for the possible PTPRA-mediated signaling pathway. Overall, the present study may provide new insight for breast cancer diagnosis and therapy.

Materials and methods

Reagents. Human recombinant TNF- α was obtained from T&L Biological Technology. Transcription factor E2F (E2F), p53, NF- κ B, eukaryotic initiation factor 2 α kinase 1 (EIK1), transforming growth factor (TGF)- β , JNK, myc proto-oncogene protein (c-MYC), PI3K/AKT, Wnt, protein giant-lens (Gil), Notch, STAT3 and ETS transcription factor (Elk1) luciferase reporter plasmids and the pHAGE puro vector were gifted by the School of life sciences, Wuhan University, Wuhan, China. These luciferase reporter constructs contain the DNA-binding motifs of transcription factor in these signaling pathways.

Analyzed datasets. PTPRA mRNA data in patients with the breast cancer gene (BRCA) were analyzed using the Gene Expression Profiling Interactive Analysis database (gepia.cancer-pku.cn) with Kaplan Meier analysis and log-rank tests as previously described (15). The expression data of 1,085 tumors and 291 adjacent normal tissues were obtained from The Cancer Genome Atlas (TCGA; www.tcg.org). Group cut-off was at 50% and based on this, patients were divided into the low PTPRA or the high PTPRA group. The overall survival was calculated in low and high PTPRA groups for 250 months according to previous reports (21-23) using the

GEPIA database to display the relevance of PTPRA mRNA in patients with breast cancer.

Cell culture. MCF-7 and HEK293T cells were purchased from the American Type Culture Collection. MCF-7 cells were cultured in DMEM/nutrient mixture F12 (1:1) (Gibco; Thermo Fisher Scientific, Inc.) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. HEK293T cells were cultured in DMEM medium supplemented with 10% FBS. All cells were cultured in a 5% CO₂ chamber at 37°C.

Construction of the FLAG-PTPRA-pGEM-T plasmid and transfection. Total RNA was extracted out using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) from MCF-7 cells and reverse transcribed into cDNA using a high capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.) at 4°C according to the manufacturer's protocols. cDNA encoding PTPRA was amplified with PCR with the following primers: Forward: 5'-GATCCGCCACCAUGGATG GATTCCTGGTTCATTCTTGTTC-3' and reverse: 5'-TCG AGCTTGAAGTTGGCATAATCTG-3'. The PTPRA fragment was gel-purified via BamH I and EcoRI and rejoined to BamH I-EcoRI digested pHAGE puro plasmid. A total of 10 μ g Flag-PTPRA expression plasmid were introduced to MCF-7 cells for exogenous overexpression using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. As the control, the empty pHAGE puro plasmid was introduced to MCF-7.

Construction of the PTPRA-deficient cell line. PTPRA knockout plasmids were constructed using the clustered regularly interspaced short palindromic repeat (CRISPR) knockout method (24,25). Briefly, the gRNA targeting sequence was obtained and inserted into CRISPR/Cas9 Plasmid using the Precision X Multiplex gRNA kit (SBI <https://systembio.com/products/crispr-cas9-systems/>) according to the manufacturer's protocol. The non-specific binding targets of the CRISPR/Cas9 plasmid served as the negative control. In total, 2.5x10⁵ HEK293T cells per well at 80-90% confluence were transfected with 400 ng CRISPR/Cas9-PTPRA plasmid or the control plasmid for 2 days. Next, MCF-7 cells were transfected with 10 μ l lentiviral particles for 72 h at 37°C. The infected MCF-7 cells were screened in the presence of 1 μ g/ml puromycin for 7 days. The puromycin-resistant cells were subjected to further confirmation by agarose gel electrophoresis and western blotting. The cells carrying the non-specific binding targets CRISPR/Cas9 plasmid were used as a control.

Western blot analysis. Collected cells were lysed with RIPA buffer (BioVision, Inc.) and the supernatant was extracted for SDS-PAGE analysis. After quantification using the Bradford assay, protein lysates (10 μ g/lane) were separated by 8% SDS-PAGE, transferred to PVDF membranes and blocked with TBS containing 5% non-fat milk at room temperature for 1 h. The membranes were probed with mouse monoclonal anti-Flag antibody (1:3,000; cat. no. 81069; ProteinTech Group, Inc.) or PTPRA antibody (1:2,000; cat. no. 13079-1-AP; ProteinTech Group, Inc.), β -actin antibody (1:2,000; cat. no. Ag27042; ProteinTech Group, Inc.) for 1-2 h at room

temperature. The blots were then incubated and reprobed with horseradish peroxidase-conjugated secondary antibody (1:2,000; cat. no. SA00001-1; ProteinTech Group, Inc.) for 1 h at room temperature. Band signals were visualized using an ECL system (GE Healthcare). The following antibodies were diluted in TBS and used for immunoblotting: Anti-FLAG, anti- β -actin and anti-PTPRA.

Colony formation, cell proliferation and Transwell cell migration assay. Overexpressed-PTPRA MCF-7 cells or two PTPRA-knockout independent clones (PTPRA^{-1#} and PTPRA^{-2#}) and their corresponding control MCF-7 cells were harvested and prepared in single-cell suspension (1×10^4 cells/ml) for the subsequent cell assays.

A colony formation experiment was conducted to estimate the clonogenic activity of breast cancer cells. Prepared cells were seeded in 6-well plates and cultured in an incubator at 37°C. Following 8-day culture, the colonies were fixed using 100% methanol for 15 min and stained using 0.5% crystal violet for 20 min at room temperature before quantification under an inverted light microscope (ECLIPSE TE2000-S; Nikon) at 200x magnification. The indicated colony formation units were recorded in 5 random fields for every replicate and plotted.

In the Cell Counting Kit (CCK)-8 assay, the aforementioned cells were cultured in 96-well plates (1×10^3 cells/well). Following incubation for 1, 3, 5 and 7 days, diluted CCK-8 solution (Dojindo Molecular Technologies, Inc.) was supplemented into each well according to the manufacturer's manual. After incubation for another 1-2 h at 37°C, cell proliferation was evaluated spectrophotometrically at a wavelength of 450 nm with an Automated Enzyme Immunoassay Analyzer (Shanghai Dongcao Biotechnology Co., Ltd, Tosoh Corporation; <https://www.biomart.cn/infosupply/57204830.htm>).

For Transwell migration assay, Transwell inserts (Corning Inc.) with porous polycarbonate membranes were firstly placed in 24-well plates. The lower compartment was filled with 2.6 ml DMEM containing 40% FBS. MCF-7 cells (1×10^4) were added to the upper compartment and cultured in Transwell plates at 37°C for 2 days. Cell debris that did not migrate through the membrane were removed with a cotton swab. The migratory cells were fixed with 5% glutaraldehyde for 10 min and stained 1% crystal violet in 2% ethanol for 20 min before images were captured and quantification under an inverted light microscope (ECLIPSE TE2000-S; Nikon). All comparison experiments were performed in triplicate.

Luciferase reporter gene assay. In order to screen the target signaling pathways of PTPRA, a series of luciferase reporter gene assays were performed to determine the effect of PTPRA on the transcriptional activity of several documented tumor signaling markers: E2F, p53, NF- κ B, EIK1, TGF- β , JnK, c-MYC, Wnt, Gil, Notch, STAT3 and Elk1.

A total of 1×10^4 HEK293T cells were cultured in 24-well plates overnight at 37°C and transfected with the aid of Lipofectamine[®] 2000 (Thermo Fisher Scientific, Inc.). Each transfection contained the indicated luciferase reporter vector (200 ng/well) and prl-tk (10 ng/well) empty control plasmid or PTPRA expression plasmid or control vector. Following 36 h incubation at 37°C, the released cells were treated with

trypsin and luciferase activity was measured using a Dual-Glo Luciferase Assay kit (Promega Corporation), according to the manufacturer's protocol.

Furthermore, HEK293T cells were transfected with pNFKB-luc and PTPRA plasmids at various diluted concentrations (100, 200 and 400 ng/well) to further confirm the effect of PTPRA on NF- κ B transcriptional activity. Luciferase activity was normalized using the *Renilla* luciferase activity.

RNA isolation and reverse transcription-quantitative PCR. Total RNA from cells was extracted from lysed cells using TRIzol[®] (Thermo Fisher Scientific, Inc.). Reverse transcription was performed using oligo dT primers using RT kit (Invitrogen) according to the manufacturer's protocol. mRNA of IKB α (one of classic downstream molecules of the NF- κ B signaling pathway (26) in and two PTPRA deficient MCF-7 cells (PTPRA^{-1#} and PTPRA^{-2#}) cells and their corresponding control MCF-7 cells (PTPRA^{+/+}) was assessed upon TNF- α stimulation or not. The relative expression was quantified by the $2^{-\Delta\Delta C_q}$ method (27).

Statistical analysis. Statistical analyses were performed using GraphPad Prism software (version 6.0; GraphPad Software). Log-rank test was carried out to calculate significance of PTPRA in predicting overall survival of breast cancer patients using GEPIA according to the creator of this website (28).

For continuous variables, measured data are presented as the mean \pm SEM. Unpaired two-tailed Student's t-test was used to compare differences between two groups. One-way ANOVA was used to evaluate the statistical significance among multiple groups followed by Tukey's post hoc corrective test. $P < 0.05$ was considered to indicate a statistically significant difference. All experiments except the analysis from GEPIA were performed at least three times.

Results

PTPRA expression and prognostic value in breast cancer. The BRCA database from GEPIA was analyzed and used to compare the expression of PTPRA in breast cancer and normal tissues in order to determine the prognostic value of PTPRA in breast cancer. PTPRA expression was significantly increased in breast cancer tissues compared with normal tissues (Fig. 1A). As presented in Fig. 1B, patients with high PTPRA demonstrated worse clinical outcomes compared with patients with PTPRA, while there was no significant difference between groups ($P = 0.45$). These results indicated that PTPRA expression level may serve a putative role in breast cancer malignancy.

PTPRA overexpression promotes proliferation, colony formation and migration of MCF-7 cells. A vector containing Flag-PTPRA was constructed and transfected into MCF-7 cells using Lipofectamine 2000 to verify the specific biological function of PTPRA. PTPRA overexpression was confirmed using anti-Flag antibodies via western blotting (Fig. 2A). Furthermore, PTPRA overexpression significantly promoted the colony formation ability of MCF cells (Fig. 2B). A growth curve analysis demonstrated that PTPRA overexpression dramatically enhanced proliferation compared with the control

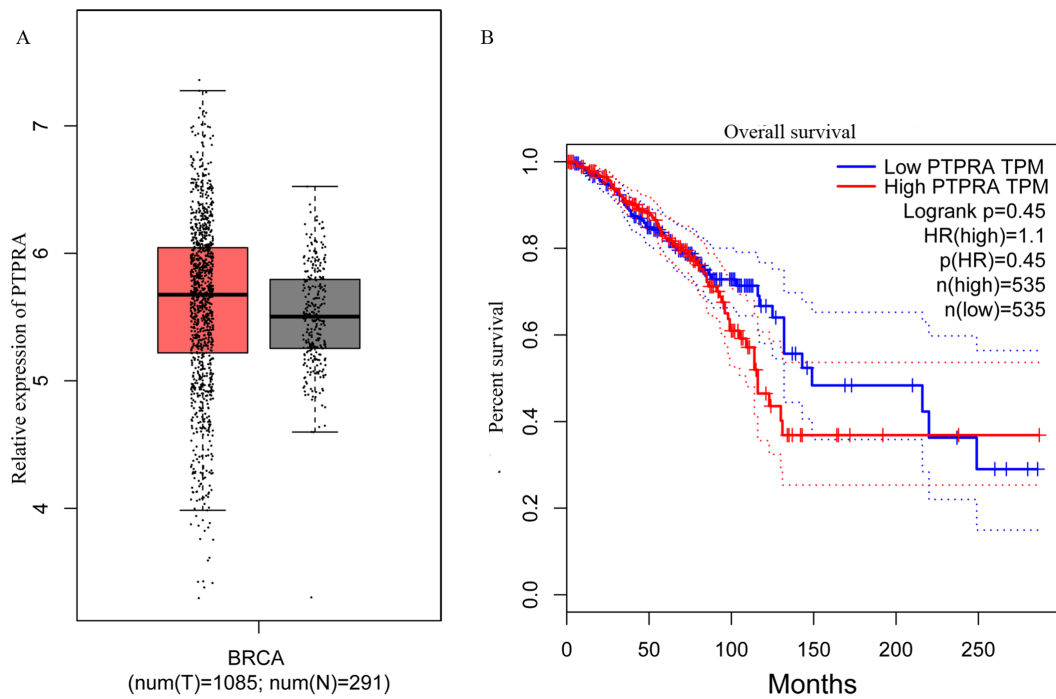


Figure 1. The PTPRA gene is highly expressed in breast cancer. (A) Relative expression level of PTPRA in 1,085 breast cancer tissues and 291 normal tissues from The Cancer Genome Atlas database. (B) Kaplan-Meier survival curves of BRCA patients with low-PTPRA and high-PTPRA in GEPIA. The blue dotted line presents patients with low PTPRA and the red dotted line represents patients with high PTPRA. PTPRA, protein tyrosine phosphatase receptor type A; HR, hazard ratio; T, tumor; N, normal; num, number; TPM, Transcripts Per Million.

cells (Fig. 2C). Additionally, the Transwell assay demonstrated that the number of migratory cells in the overexpression group was significantly increased compared with the control group (Fig. 2D). These results indicated that PTPRA increased the tumorigenic properties of MCF7 cells *in vivo*.

Knockout of PTPRA suppresses the proliferation and migration ability of MCF-7 cells *in vitro*. PTPRA was further depleted in MCF-7 cells using CRISPR to confirm the effect of PTPRA on cell behaviors. Western blotting revealed that PTPRA was almost completely silenced (Fig. 3A). Furthermore, the colony formation ability and proliferation of PTPRA-deficient MCF-7 cells were significantly decreased compared with that of control MCF-7 cells (PTPRA^{+/+}) (Fig. 3B and C, respectively). Consistently, MCF7 cells deficient in PTPRA had fewer migratory cells than the control cells (PTPRA^{+/+}) (Fig. 3D). These results suggested that PTPRA deficiency decreased cell colony formation ability and inhibited tumor cell migration ability.

Signaling pathway is regulated by PTPRA in breast cancer. Oncogenesis, the development and prognosis of tumors, involves complicated pathway networks that are implicated in numerous signal pathways, such as microtubule-associated protein kinase, NF- κ B and signal transducer and activator of transcription factor 3 (29). The results of luciferase reporter gene assays demonstrated that NF- κ B transcriptional activity was markedly increased compared with controls (Fig. 4A). The luciferase reporter gene assay did not demonstrate any obvious alterations in the expression of the other signaling molecules. NF- κ B transcriptional activity was also demonstrated to be increased in a dose-dependent manner (Fig. 4B). These results indicated

that PTPRA overexpression in HEK293T cells stimulated NF- κ B transcriptional activity.

TNF α -activates NF- κ B in MCF-7 cells. RT-qPCR was utilized to quantify one of the classic downstream molecules of the NF- κ B signaling pathway, I κ B α in MCF-7 cells. No transcription of the I κ B α gene was detected in PTPRA^{-/-} and PTPRA^{+/+} MCF-7 cells without TNF- α treatment (Fig. 4C). However, the TNF- α stimulus changed these outcomes. I κ B α gene transcription in PTPRA^{+/+} MCF-7 cells was increased compared with that in PTPRA-deficient MCF-7 cells. These outcomes indicated that TNF- α -mediated PTPRA stimulated the activation of NF- κ B and promoted the tumor phenotype of breast cancer cells.

Discussion

PTPRA is closely associated with neoplastic transformation through its effects on proliferation and migration in breast cancer cells (30). However, the oncogenic characteristics of PTPRA remain elusive *in vitro*. The present study demonstrated the significance of PTPRA on the migration and metastatic potential of MCF-7 breast cancer cells. Additionally, to the best of our knowledge, these results are the first to reveal that PTPRA may act as a proto-oncogene in the TNF- α -dependent inflammatory responses by directly binding to NF- κ B *in vitro*.

The results of the present study demonstrated that PTPRA expression was significantly increased in the tumor tissues of patients with breast cancer compared with normal tissues via analysis of TCGA data from GEPIA. The GEPIA dataset also suggested that patients with breast cancer exhibiting high expression levels of PTPRA and slightly worse clinical outcomes

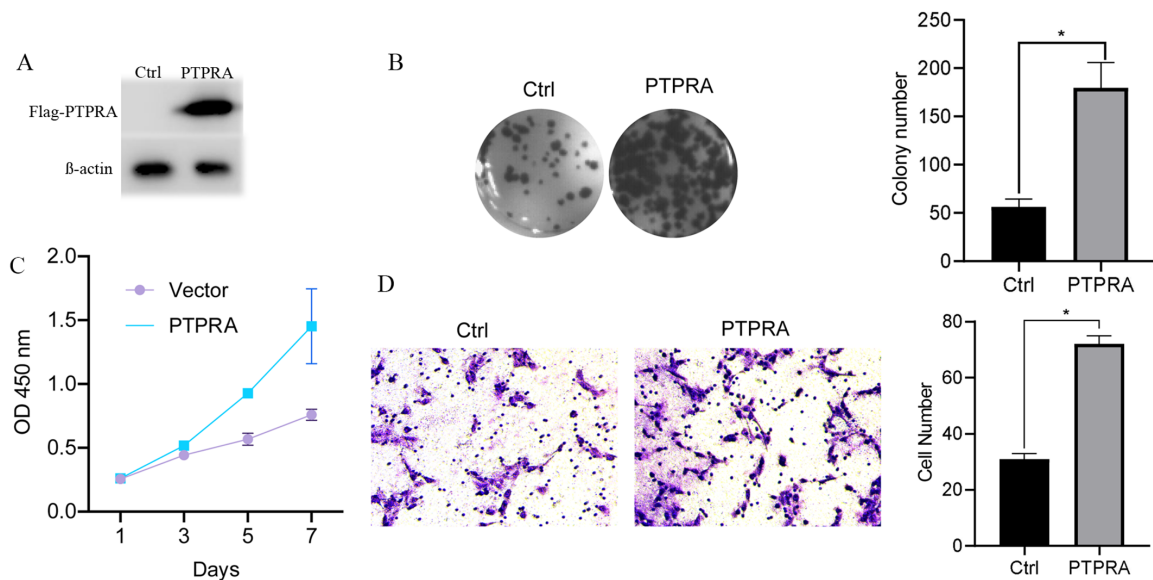


Figure 2. PTPRA overexpression promotes proliferation, colony formation and migration in breast cancer cells. (A) MCF-7 cells were successfully transfected with FLAG-PTPRA vector. Western blotting was performed to evaluate exogenous PTPRA expression in wild-type and PTPRA-overexpressed MCF-7 cells with FLAG antibodies. (B) A clonogenic formation assay was performed to determine the effect of PTPRA overexpression in MCF-7 cells. Representative images are displayed. (C) Cell proliferation was assessed using a Cell Counting Kit-8 assay in control and PTPRA-overexpressing MCF-7 cells. (D) PTPRA overexpression enhanced migration in breast cancer cells compared with the empty vector group. * $P < 0.05$ vs. respective Ctrl. PTPRA, protein tyrosine phosphatase receptor type A; Ctrl, control; OD, optical density.

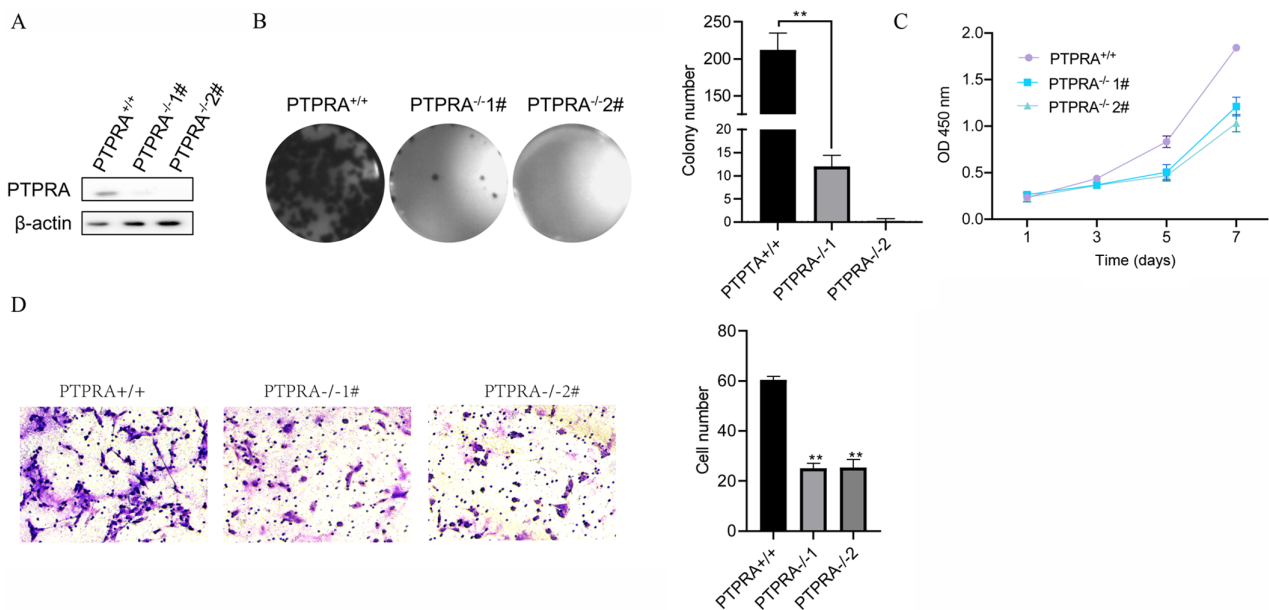


Figure 3. PTPRA deficiency inhibited colony formation and migration in breast cancer cells. (A) Expression of recombinant wild-type PTPRA and PTPRA knockout in MCF-7 cells was measured by western blotting. (B) Colony formation assay was performed to determine the effect of PTPRA knockdown in MCF-7 cells. (C) Cell proliferation of PTPRA^{+/+} and PTPRA^{-/-} MCF-7 cells. (D) PTPRA deficiency suppressed migratory capacity in breast cancer cells compared with wild-type MCF-7 cells. PTPRA, protein tyrosine phosphatase receptor type A; OD, optical density. ** $P < 0.01$ vs. PTPRA^{+/+} MCF-7 cells.

when compared with low-PTPRA patients, though the difference was not statistically significant. These results revealed that PTPRA acts as an enhancer of tumorigenicity and increases the malignancy of breast cancer types. In the present study, clonogenic and migratory behaviors in PTPRA-overexpressing or PTPRA-deficient breast cancer cell lines were investigated. The results were consistent with a recent study that demonstrated that PTPRA accumulation in MCF-7 cells facilitates focal

adhesion formation and cell migration *in vitro* (17), indicating that PTPRA may be a pro-migratory factor. Furthermore, a retrospective cohort analysis demonstrated PTPRA overexpression in squamous cell lung cancer (19). PTPRA overexpression promotes lung cancer cell proliferation and is associated with worse overall survival, suggesting that PTPRA overexpression may be an effective predictive or prognostic marker for squamous cell lung cancer (19).

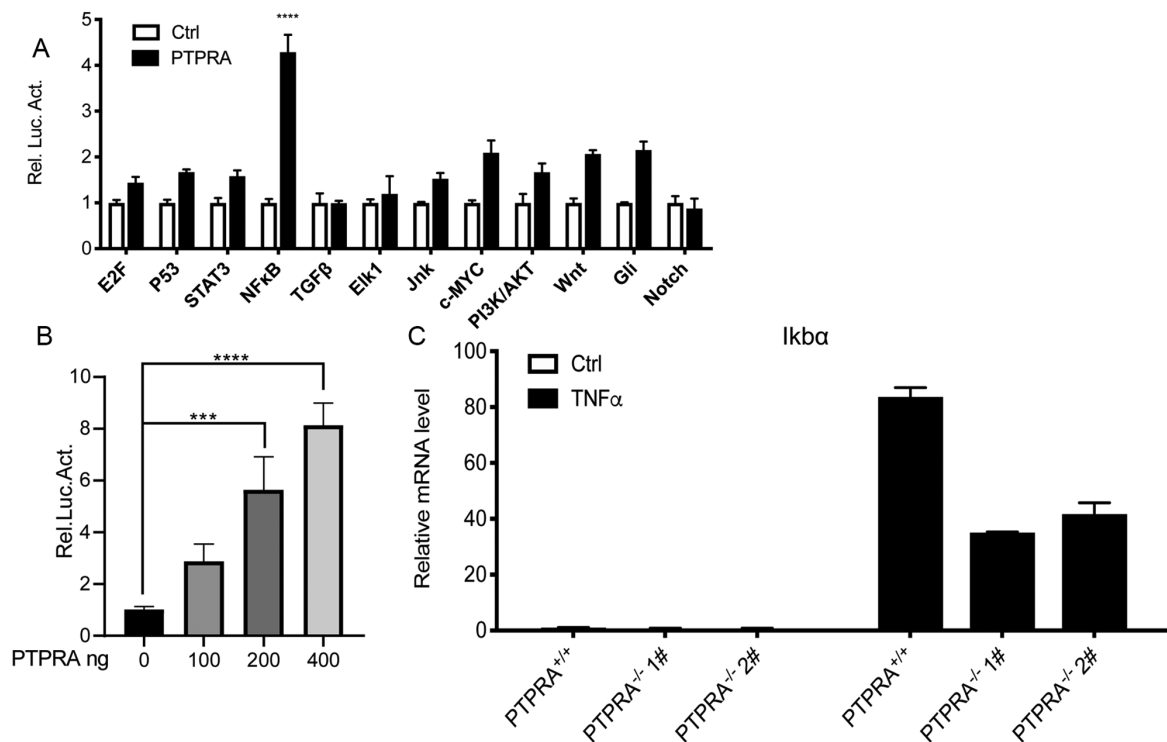


Figure 4. Overexpression of PTPRA contributes to an enhanced inflammatory response in MCF-7 breast cancer and HEK293T cells. (A) Screening of human cancer pathways demonstrated that PTPRA markedly enhanced NF- κ B transcriptional activity in HEK293T cells. (B) NF- κ B activity was increased in a PTPRA-dependent manner as detected by luciferase assays in MCF-7 cells. (C) Reverse transcription-quantitative PCR analysis was performed to examine the expression level of NF- κ B-targeted gene followed by TNF- α stimulation. *** $P < 0.001$, **** $P < 0.0001$ vs. PTPRA^{+/+} MCF-7 cells. PTPRA, protein tyrosine phosphatase receptor type A; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; TNF- α , tumor necrosis factor α ; Rel. Luc. Act., relative luciferase activity; STAT3, signal transducer and activator of transcription 3; TGF- β , transforming growth factor β ; Jnk, c-Jun N-terminal kinase; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; E2F, transcription factor E2F; c-MYC, myc proto-oncogene protein; Gli, protein giant-lens.

Protein phosphatases are critical modulators of cell signaling. Their functional roles in aberrant signaling are critical for tumor pathogenesis. PTPRA, one of the classic PTPs, executes its signaling functions primarily through directly dephosphorylating key signaling molecules or activating the oncogenic focal adhesion kinase-Src complex in breast cancer cells (6,31). Furthermore, a previous study using an animal model of pulmonary fibrosis has revealed that PTPRA directly interacts with mothers against decapentaplegic homolog (Smad) protein and increases Smad transcriptional activity in response to TGF- β stimuli, indicating that PTPRA has a profound effect on the genesis of inflammatory pulmonary fibrosis (32), which lead to the present study investigating the detailed information regarding the oncogenic action of PTPRA. The present study utilized a series of luciferase pathway screening assays and the results revealed that alterations in the inflammatory NF- κ B signaling pathway were largest compared with those of other oncogenic signaling pathways. Furthermore, the NF- κ B inflammation signaling pathway was activated by TNF- α stimulus, an extensively used approach to assess the mediation of target protein to certain signaling pathways, such as PI3K/AKT signaling (33-37), in order to further validate the regulatory function of PTPRA. These results indicated an oncogenic role of PTPRA in the TNF- α -induced inflammatory pathway, which has also been linked to the inflammogenesis of breast cancer (38). Ghandadi *et al* (39) reported similar results by demonstrating that the treatment of MCF-7 cells with TNF- α triggered activation of NF- κ B, ultimately leading to

receptor-interacting serine/threonine-protein kinase 1 ubiquitination and non-apoptotic death.

Activation of NF- κ B is a crucial event which supports chronic inflammation and cancer progression (40). Previous studies have demonstrated that the NF- κ B pathway may exert a number of roles in different settings or cellular contexts. In enterocytes, NF- κ B has been implicated in tumorigenesis; however, it has not been implicated in cancer progression or growth (41). These results were supported by Ardini *et al* (10), indicating that PTPRA is positively correlated with low tumor grade. The present study also supports the hypothesis that PTPRA is a downstream target of TNF- α and triggers the genesis of breast tumors (42). In the present study, TNF- α stimuli contributed to a significant PTPRA upregulation in PTPRA^{+/+} MCF-7 cells compared with PTPRA^{-/-} MCF-7 cells, indicating that crosstalk between PTPRA and TNF- α activates downstream signaling (43). Hence, it is essential to determine to what extent PTPRA influences breast cancer by TNF- α -induced NF- κ B activation.

To date, there has been compelling evidence that PTPRA is responsible for Src tyrosine 530 dephosphorylation, which leads to cellular transformation (19,44,45). For instance, Lai *et al* (44) confirmed that PTPRA overexpression activated pp60c-src kinase *in vitro* and *in vivo*, which contributed to cellular transformation and induced lung tumorigenesis *in vivo*. In the present study, the results demonstrated that PTPRA directly bound to an NF- κ B promoter and enhanced its transcriptional activity, promoting the clonogenic and

migratory tumor phenotype of breast cancer. We also noticed that PTPRA can increased the expression level of IKB α :one of classic downstream molecules of the NF- κ B signaling pathway. Whether c-Src is one of the signaling checkpoints in this signaling pathway is yet to be determined. Li *et al* (46) reported that TNF- α triggered two parallel, but independent, signaling pathways (Src and TNF receptor 1/NF- κ B) to regulate neuroses in the mouse fibrosarcoma L929 cell line. Another previous study supported the notion that a cloned osteoclastic protein-tyrosine phosphatase (PPT-oc) enhances osteoclast activity partially via the PPT-oc/c-Src/NF- κ B signaling pathway (47). These diverse signaling networks may explain the dual roles that PTPRA serves in breast cancer. In future studies, whether c-Src is a crucial participant in these regulatory mechanisms will be investigated.

There are limitations in the current study that need to be noted. The main limitation is that a single breast cancer cell line MCF-7 was used. Future studies should focus on more breast cancer cell lines, which will further elucidate the underlying mechanisms of PTPRA in breast cancer. Another limitation is that only the level of IKB α mRNA in MCF-7 cell lines was assessed following the screening of underlying oncogenic signaling pathways in HEK293T cells, further systematic approaches, including chromatin immunoprecipitation, mutational experiments and *in vivo* assays, should be performed to further validate results. Additionally, vectors that overexpressed multiple genes were generated in our lab, therefore flag antibody was used to screen the proposed-gene overexpressing cells. But only PTPRA overexpression was performed in this study.

In conclusion, the present study demonstrated that PTPRA is upregulated in patients with breast cancer. The oncogenic gene *PTPRA* is mediated by TNF- α and may partially activate the NF- κ B inflammation signaling pathway in MCF-7 breast cancer cells. These results further elucidate the function of PTPRA in breast cancer and indicate that PTPRA may be an effective diagnostic curative target for breast cancer.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and GEPIA repository (gepia.cancer-pku.cn).

Authors' contributions

FZ designed the study. CL contributed to analysis and manuscript preparation. SX performed data analysis and wrote the manuscript. XH provided assistance for data acquisition, data analysis and statistical analysis and constitutive analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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