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PTP1B Suppresses Prolactin Activation of Stat5 in Breast Cancer Cells

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Basal levels of nuclear localized, tyrosine phosphorylated Stat5 are present in healthy human breast epithelia. In contrast, Stat5 phosphorylation is frequently lost during breast cancer progression, a finding that correlates with loss of histological differentiation and poor patient prognosis. Identifying the mechanisms underlying loss of Stat5 phosphorylation could provide novel targets for breast cancer therapy. Pervanadate, a general tyrosine phosphatase inhibitor, revealed marked phosphatase regulation of Stat5 activity in breast cancer cells. Lentiviral-mediated shRNA allowed specific examination of the regulatory role of five tyrosine phosphatases (PTP1B, TC-PTP, SHP1, SHP2, and VHR), previously implicated in Stat5 regulation in various systems. Enhanced and sustained prolactin-induced Stat5 tyrosine phosphorylation was observed in T47D and MCF7 breast cancer cells selectively in response to PTP1B depletion. Conversely, PTP1B overexpression suppressed prolactin-induced Stat5 tyrosine phosphorylation. Furthermore, PTP1B knockdown increased Stat5 reporter gene activity. Mechanistically, PTP1B suppression of Stat5 phosphorylation was mediated, at least in part, through inhibitory dephosphorylation of the Stat5 tyrosine kinase, Jak2. PTP1B knockdown enhanced sensitivity of T47D cells to prolactin phosphorylation of Stat5 by reducing the EC_{50} from 7.2 nmol/L to 2.5 nmol/L. Immunohistochemical analyses of two independent clinical breast cancer materials revealed significant negative correlations between levels of active Stat5 and PTP1B, but not TC-PTP. Collectively, our data implicate PTP1B as an important negative regulator of Stat5 phosphorylation in invasive breast cancer. ***(Am J Pathol 2010, 177:2971–2983; DOI: 10.2353/ajpath.2010.090399)***

In breast epithelia, the transcription factors Stat5a and Stat5b (hereafter collectively termed Stat5) mediate prolactin-induced growth and differentiation. During pregnancy and lactation, the prolactin/Jak2/Stat5 pathway is critical for lobuloalveolar expansion, maintenance of a terminally differentiated epithelium, and the induction of major milk-related genes such as whey-acidic protein and β-casein.[1–6] Following tyrosine phosphorylation by the prolactin receptor-associated kinase Jak2, Stat5 translocates to the nucleus and binds target DNA sequences. Outside of pregnancy and lactation, a basal level of nuclear localized, tyrosine phosphorylated Stat5 is present in healthy human breast epithelia.[2] In contrast, Stat5 remains expressed but is frequently unphosphorylated in human breast cancer, a finding that is correlated with higher tumor grade, metastatic progression, and poor clinical outcome.[7–10] Furthermore, experimental evidence supports a prodifferentiation and invasion-suppressive role for prolactin/Jak2/Stat5 signaling in breast cancer.[11–13] Therefore, identifying the mechanisms underlying Stat5 dephosphorylation and potential transcriptional inactivation in breast cancer could provide novel therapeutic targets for breast cancer therapy.

Several negative regulators of the prolactin/Jak2/Stat5 pathway have been identified. SOCS1, SOCS3, and CIS

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bind Stat5 and decrease activity, block docking sites, and/or target various proteins in the pathway for proteasomal degradation.[14–18] Caveolin-1 has been reported to suppress Jak2/Stat5 signaling in mouse mammary epithelia,[19] and PIAS3 inhibits Stat5 through sumoylation and degradation.[20–22] However, the absence of Stat5 tyrosine phosphorylation in the presence of continued Stat5 protein expression in clinical breast cancer specimens suggests that tyrosine phosphatases are important regulators. In this study we focused on identifying tyrosine phosphatases that negatively regulate Stat5 tyrosine phosphorylation in human breast cancer.

A recent review noted limitations of previous studies of phosphatase regulation of prolactin-induced Jak2-Stat5 phosphorylation, most notably that no studies have been performed in the context of human breast cancer, and the few studies that used normal mammary epithelial cells relied exclusively on overexpression methodology.[23] To overcome and resolve these deficiencies we took a candidate approach focused on gene knockdown strategies to specifically identify tyrosine phosphatases that negatively regulate Stat5 phosphorylation in breast cancer. There are 107 phosphatases in the human proteome capable of dephosphorylating tyrosine residues.[24] Of these, the classical tyrosine phosphatases PTP1B, TC-PTP, SHP1, and SHP2 and the dual specificity phosphatase VHR were chosen for investigation based on their reported regulation of Stat5 activity in non-breast cells and tissues, modulation of Jak2/Stat5 homologues signaling in lower organisms, or ability to regulate Stat5 using *in vitro* overexpression systems and phosphatase assays.

There are four Jak and seven Stat genes in mammals but only one Jak (hop) and one Stat (Stat92E) in *Drosophila*.[25] Two recent studies using systemic RNAi strategies screened the *Drosophila* genome and independently identified the *Drosophila* phosphatase Ptp61F as a negative regulator of hop/Stat92E.[26,27] Ptp61F overexpression can suppress melanotic tumors,[27] a finding consistent with the oncogenic role of Stat5 in hematopoietic malignancies in humans.[28–38] There are two mammalian homologues of Ptp61F, TC-PTP, and PTP1B. Overexpression studies in Cos7 and the untransformed mouse mammary cell line COMMA-1D indicated that both PTP1B and TC-PTP suppress prolactin-induced phosphorylation of Stat5.[39,40] In contrast, studies performed using TC-PTP (−/−) MEFs or TC-PTP overexpression in the growth hormone system were unable to show effects on Stat5 tyrosine phosphorylation.[41,42] Furthermore, although PTP1B has been implicated in the regulation of Jak2 in the growth hormone,[42,43] leptin,[44–47] and interferon[48] pathways, prolactin-induced Jak2 phosphorylation was not affected by PTP1B in Cos7 or COMMA-1D cells.[40]

SHP1 has been reported as a likely candidate for dephosphorylation of growth hormone-activated Stat5.[49] SHP1 is commonly inactivated in a majority of leukemia and lymphomas,[50] cancers where Stat5 has a significant proliferative role.[28–38] SHP1 is also expressed in epithelial cells[51] and is lost in estrogen receptor (ER)-negative breast cell lines and in some colorectal cell lines.[52,53] SHP2 is a more ubiquitously expressed phosphatase than SHP1. Substrate trapping mutants have indicated that SHP2 but not SHP1 interacts with Stat5 in overexpression systems.[49,54] *In vitro* tyrosine phosphatase assays and indirect immunofluorescence have indicated the formation of a complex between SHP2 and Stat5.[55] Interestingly, SHP2 phosphatase activity was required for maintaining the activated state of Stat5 as opposed to its dephosphorylated form. Further evidence that SHP2 positively regulates the Jak2/Stat5 pathway is based on findings that SHP2 can decrease the amount of SOCS1 mediated Jak2 degradation[56] and that SHP2 can form a complex between the GHR and Jak2.[57] Consistent with these findings, SHP2 is also a positive regulator of interferon signaling.[58] The dual-specificity phosphatase VHR, in contrast, was recently implicated as a negative regulator of Stat5 in interferon signaling. VHR acted as part of a regulatory loop whereby interferon-α/β-activated Tyk2 phosphorylated and activated VHR, leading to specific nuclear dephosphorylation of phosphotyrosine-Stat5.[59]

Based on these variable and inconsistent observations from overexpression studies, we used gene knockdown using specific shRNA to query the importance of each of five candidate phosphatases in enhancing prolactin-induced Jak2-Stat5 phosphorylation in human breast cancer cells. Collectively, our data from knockdown studies *in vitro* and correlation analyses of clinical breast specimens indicate that dysregulation of PTP1B represents a major mechanism contributing to the frequent loss of Stat5 phosphorylation in invasive breast cancer.

Materials and Methods

Antibodies and Reagents

Human prolactin was provided by Dr. A.F. Parlow under the sponsorship of the National Hormone and Pituitary Program (Torrance, CA). Monoclonal mouse anti-phosphotyrosine-Stat5 antibody (AX1) and polyclonal rabbit anti-Stat5a (AX551) and anti-Stat5b (AX554) were obtained from Advantex BioReagents (Conroe, TX). Mouse monoclonal pan-Stat5, SHP2, VHR, and anti-mouse and anti-rabbit horseradish peroxidase-conjugated secondary antibodies were purchased from BD Biosciences (Bedford, MA). Mouse monoclonal Jak2 was purchased from BioSource (Camarillo, CA). Rabbit polyclonal Jak2 antibody, phosphotyrosine (4G10) mouse monoclonal, and PTP1B rabbit polyclonal were purchased from Upstate Biotechnology (Lake Placid, NY). Lamin A/C rabbit polyclonal antibodies were purchased from Cell Signaling. Rabbit polyclonal calnexin was obtained from Stressgen (Ann Arbor, MI). SHP1, PTP1B (PHO2), and TC-PTP (PHO3–CF4 clone) mouse monoclonal antibodies were purchased from Calbiochem. Monoclonal mouse anti-Jak2 was purchased from BioSource. Sodium orthovanadate and catalase from bovine liver were purchased from Sigma. Hydrogen peroxide 30% was purchased from Fisher Scientific and the luciferase assay system was purchased from Promega. Protein A-Sepharose beads were purchased from Amersham Biosciences.

Cell Culture

The human breast cancer cell lines T47D and SKBr3 (ATCC, Manassas, VA) were grown in RPMI 1640 medium (Biofluids, Rockville, MD) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 2 mmol/L L-glutamine (GIBCO), and 1 mmol/L sodium pyruvate (Cellgro). The human breast cancer cell lines MCF-7 and MDA-MB-231 (ATCC) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Atlanta Biologicals). Human breast cancer cell line ZR-75-1 (ATCC) was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 1 mmol/L sodium pyruvate (Cellgro). The stably transfected T47D-βCASLUC cell line (previously described 60) was maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Atlanta Biologicals), 2 mmol/L L-glutamine (GIBCO), 1 mmol/L sodium pyruvate (Cellgro), and 0.5 μg/ml puromycin. The 293FT cells (Invitrogen) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Atlanta Biologicals), 2 mmol/L L-glutamine (GIBCO), 1 mmol/L sodium pyruvate (Cellgro), 1X MEM nonessential amino acids (GIBCO), and 500 μg/ml G418.

Whole Cell Lysates, Immunoblots, and Immunoprecipitations

For whole cell lysates, cells were washed with cold PBS, lysed in lysis buffer (50 mmol/L Tris pH 7.5, 150 mmol/L NaCl, 0.1% SDS, 1% Igepal, 0.5% sodium deoxycholate, 2 mmol/L EDTA, 0.01 mol/L sodium phosphate, 50 mmol/L NaF, 2 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonylfluoride, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin), collected by scraping and transferred to Microfuge tubes, and rotated for 1 hour at 4°C end-over-end. Insoluble material was pelleted at 13,000 $\times$ *g* at 4°C for 30 minutes. Protein concentration was determined by the BCA protein assay kit (Pierce). Standard immunoblot procedures were followed. Briefly, 10 or 15 μg of total protein was resolved on a NuPAGE 4 to 12% Bis-Tris gradient gel (Invitrogen). Proteins were transferred onto a polyvinylidene difluoride membrane, the membrane was blocked for 1 hour at ambient temperature with 1% bovine serum albumin, and subsequently probed with antibody at 4°C overnight. The membranes were then washed, probed with anti-mouse secondary antibody, washed, and visualized by chemiluminescence. Phosphotyrosine immunoblots were stripped and reprobed for total protein expression. For immunoprecipitation, clarified whole cell lysates were incubated with indicated antibodies for 2 hours at 4°C. Antibody-protein complexes were captured by incubation with Protein A-Sepharose beads (Amersham Biosciences, Inc., Piscataway, NJ) rotating at 4°C for 1 hour and washed three times in 1 ml of lysis buffer. Immunoprecipitated proteins were dissolved in 2X loading buffer containing reducing agent, heated to 70°C for 10 minutes, resolved by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes. Immunoblotting was performed as described above.

Lentivirus Production and Infections

The lentiviral packaging plasmid pCMV-dR8.2 dvpr (plasmid 8455) and envelope plasmid pCMV-VSV-G (plasmid 8454) were from Addgene. pLKO.1-puro lentiviral vectors containing PTP1B shRNA no. 1 (catalog no. RHS3979-9571386), PTP1B shRNA no. 2 (catalog no. RHS3979-9571385), TC-PTP shRNA no. 1 (catalog no. RHS3979-9571389), TC-PTP shRNA no. 2 (catalog no. RHS3979-9571387), SHP1 shRNA no. 1 (catalog no. RHS3979-9575809), SHP1 shRNA no. 2 (catalog no. RHS3979-9575811), SHP2 shRNA no. 1 (catalog no. RHS3979-9573790), SHP2 shRNA no. 2 (catalog no. RHS3979-9573787), VHR shRNA no. 1 (catalog no. RHS3979-9575758), VHR shRNA no. 2 (catalog no. RHS3979-9575761), and non-target control shRNA (catalog no. SHC002) were purchased from Open Biosystems. Lentiviral particle production was achieved by cotransfecting a T175 flask containing 95% confluent 293FT cells (Invitrogen) with 9 μg of shRNA lentiviral plasmid along with 24 μg of pCMV-dR8.2 dvpr and 2.4 μg of pCMV-VSV-G (a 10:1 ratio). Transfections were carried out using 108 μl of Lipofectamine 2000 (Invitrogen), and virus was harvested 48 to 72 hours after transfection. Lentiviral knockdown of indicated proteins was achieved by infecting 90% confluent cells with lentivirus overnight in the presence of 8 μg/ml Polybrene. The infection medium was then replaced with fresh growth medium and cultured for 56 to 72 hours to allow for efficient protein knockdown. Optimal amount of virus to treat cells was determined by dose curve and immunoblot analysis with subsequent experiments performed using the lowest amount of virus that gave the maximum amount of knockdown.

Sodium Orthovanadate Treatment of Cells

A 1-ml solution of 6 mmol/L sodium orthovanadate in PBS was treated with 6 μl of 3% H_2O_2, vortexed, and incubated for 5 minutes at ambient temperature. Inactivation of excess H_2O_2 was accomplished by the addition of 1000 units of catalase from bovine liver for 2 minutes at ambient temperature. Activated inhibitor was immediately used to treat cells. Briefly, confluent T47D cells were serum-starved for 16 hours, then pretreated for 10 minutes in fresh serum-free medium with or without 120 μmol/L pervanadate followed by stimulation with 10 nmol/L prolactin for 20 minutes in fresh serum-free medium with or without fresh 120 μmol/L pervanadate. Prolactin-containing media was washed off and replaced with fresh serum-free medium with or without fresh 120 μmol/L pervanadate, and total cell lysates were collected at the indicated time points and phosphotyrosine-Stat5 status was monitored by immunoblotting.

Time Course Experiments

T47D and MCF7 cells were infected with lentivirus expressing indicated shRNA constructs for 56 hours. Cells were serum starved overnight, then treated with a pulse of 10 nmol/L prolactin in fresh serum-free medium for 20 minutes. Prolactin-containing medium was replaced with fresh serum-free medium and cells were harvested at time points as indicated using either the whole cell lysate or cellular fractionation protocols described and analyzed by immunoblotting. For PTP1B overexpression studies, 293FT cells were plated in six-well plates and transfected at 90% confluency with 3.9 μg total DNA (1.3 μg of pcDNA3-PrlR, 1.3 μg of pDNRStat5, and 1.3 μg of pcDNA3.1/Zeo+/PTP1B or empty vector control) using 10 μl of Lipofectamine 2000 according to the manufacturer's instructions. Fifty-six hours after transfection, cells were serum-starved for 16 hours, stimulated with 10 nmol/L prolactin for 15 minutes, and cultured in fresh medium for the indicated times. Cell lysates were analyzed by immunoblotting as described above and densitometry was conducted from three or four experiments.

Cell Fractionation

Lentiviral-infected, prolactin-stimulated MCF7 cells were washed with cold PBS twice and then scraped in PBS. Cells were pelleted at 800 $\times$ *g* and resuspended in buffer A (10 mmol/L HEPES pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.4% Igepal, 2 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonylfluoride, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin). Cells were incubated for 10 minutes on ice, centrifuged at maximum speed for 3 minutes, and the supernatant was saved as the cytosolic fraction. The remaining pellet was washed once with cold PBS and resuspended in buffer B (20 mmol/L HEPES pH 7.9, 200 mmol/L NaCl, 1 mmol/L EDTA, 10% glycerol, 2 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonylfluoride, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, and 2 μg/ml leupeptin). The cell suspension was shaken vigorously for 2 hours at 4°C, centrifuged at maximum speed for 5 minutes, and the supernatant saved as the nuclear fraction. Protein concentrations and immunoanalyses were performed as described above.

Luciferase Assays

T47D-βCASLUC cells were infected with lentivirus in a 24-well plate, and 72 hours after infection were serum-starved with or without 10 nmol/L prolactin. After 8 hours of stimulation the cells were harvested in 1X passive lysis buffer, and luciferase activity was measured using luciferase reporter assay system (Promega, Madison, WI). Raw luciferase values ($\pm$SD) were graphed. Three experiments were performed in triplicate with an extra well of cells plated for each lentiviral treatment condition to confirm effective knockdown. Whole cell lysates were prepared as described above and immunoblot analysis was performed to determine extent of protein knockdown.

Dose-Response Experiments

Lentiviral-infected T47D cells were serum-starved 56 hours after infection, serum-starved overnight, and stimulated in fresh serum-free medium with one of seven different prolactin doses for 15 minutes or left unstimulated as a control. Densitometry was performed from three experiments, and EC_{50} was calculated using SigmaPlot.

Immunohistochemistry

Analyses were performed on a CEMA array containing 48 nonmalignant and 47 malignant breast tissues (material I). Malignant tissues were represented by ductal carcinoma *in situ* (n = 3), grade 1 invasive ductal carcinoma (n = 5), grade 2 invasive ductal carcinoma (n = 22), and grade 3 invasive ductal carcinoma (n = 17). Among the invasive ductal carcinomas, 68% were ER-positive, 58% were progesterone receptor-positive, and 13% were Her2-positive. The array sections were rehydrated, heated for 30 minutes in citrate buffer pH 6 (DAKO) in a microwave for antigen retrieval, blocked with horseradish peroxidase for 15 minutes, washed, blocked with 10% goat serum for 30 minutes, and incubated in primary antibodies to phosphotyrosine-Stat5, PTP1B, or TC-PTP overnight at 4°C. The array was then washed, treated with SS Link solution (BioGenex, San Ramon, CA) for 20 minutes, washed, treated with SS Label solution (BioGenex) for 20 minutes, washed, and stained with diaminobenzidine. To complete the protocol the array was counterstained with hematoxylin, dehydrated, and mounted with a coverslip. Staining for PTP1B in nonmalignant and malignant breast epithelia was generally homogenous, and pathologists scoring focused on overall staining intensity instead of Allred score. The stained array was scored by an independent pathologist as 0, +, ++, +++, and statistical analyses were performed using SPSS.

Automated Quantitative Analysis (AQUA)

T47D cells were treated with increasing doses of lentivirus delivering PTP1B shRNA and allowed to culture for 72 hours for efficient knockdown of PTP1B. Cells were fixed, collected by low-speed centrifugation, and resuspended in HistoGel for sectioning, fluorescent immunostaining, and AQUA analysis for detection of PTP1B expression. Quantitative analysis of PTP1B and phosphotyrosine-Stat5 expression was performed on a second clinical material, material II, represented by a CEMA tissue array containing 100 primary invasive breast carcinoma specimens (grade 1, n = 20; grade 2, n = 40; grade 3, n = 40). Hormone receptor status as determined by standard IHC identified 59% ER-positive, 42% progesterone receptor-positive, and 20% Her2-positive specimens. Slides were deparaffinized, subjected to low pH antigen retrieval using the Dako PT module, and stained on a Dako Autolink Plus autostainer. Endogenous peroxidase activity was blocked using Dako FLEX peroxidase block for 10 minutes followed by serum-free protein block for 30 minutes. Slides were incubated for 20 minutes with a

primary antibody mixture of rabbit anti-phosphotyrosine-Stat5 (Epitomics 1208-1; 1:1600) and mouse anti-cytokeratin (Dako M3515; 1:100), or mouse anti-PTP1B (Cell Signaling PHO2; 1:400) and rabbit anti-cytokeratin (Dako Z0622; 1:100), washed three times with Dako wash buffer, and incubated with relevant secondary antibodies. The secondary antibody cocktail included anti-rabbit horseradish peroxidase and anti-mouse Alexa Fluor 488 conjugate (Molecular Probes; 1:200) for phosphotyrosine-Stat5 staining, or anti-mouse horseradish peroxidase and anti-rabbit Alexa Fluor 488 conjugate (Molecular Probes; 1:200) for PTP1B staining. Following incubation the slides were washed three times with Dako wash buffer and incubated with tyramide-Cy5 (Perkin-Elmer). Finally, all sections were stained with 4′,6-diamidino-2-phenylindole for nuclear visualization. Automated quantitative analysis was performed using the PM2000 imaging platform and AQUA software (HistoRx) and statistical analysis performed using SPSS.

Results

Prolactin-Induced Tyrosine Phosphorylation of Stat5 in T47D Cells Is Enhanced and Prolonged by the Tyrosine Phosphatase Inhibitor Pervanadate

To broadly assess the involvement of tyrosine phosphatases as negative regulators of Stat5 signaling in human breast cancer cells, we first determined the effect of the unselective tyrosine phosphatase inhibitor pervanadate. T47D cells were pretreated with 120 μmol/L pervanadate for 10 minutes, stimulated with prolactin for 20 minutes followed by the replacement of prolactin-containing medium with pervanadate-containing or control medium, and monitored over time. Pervanadate treatment did not affect basal phosphorylation levels of Stat5 but did result in a markedly enhanced prolactin-induced phosphotyrosine-Stat5 signal that remained present over the entire 2-hour time course (Figure 1A). In contrast, control T47D cells not exposed to pervanadate showed weaker and more transient phosphotyrosine-Stat5 induction with levels of phosphotyrosine-Stat5 returning to near baseline by 60 minutes. These initial data verified that tyrosine phosphatases are actively involved in negatively regulating prolactin-induced phosphotyrosine-Stat5 in T47D breast cancer cells.

PTP1B, TC-PTP, SHP1, SHP2, and VHR Are Expressed in Breast Cancer Cell Lines

Based on studies performed in model systems other than normal or malignant human breast, we determined whether five candidate phosphatases implicated in the regulation of Stat5 activity were expressed in a panel of human breast cancer cell lines. Figure 1B shows that PTP1B, TC-PTP, SHP1, SHP2, and VHR are ubiquitously expressed across the various cell lines but at variable levels. The lowest level of expression was SHP1 in MDA-MB-231 cells where SHP1 was detectable in immunoblots only after overexposure of film (not shown). Based on the broad expression of the candidate phosphatases in T47D cells and its high level of prolactin receptors, T47D cells were chosen as the primary breast cancer model cell line for the subsequent experiments.

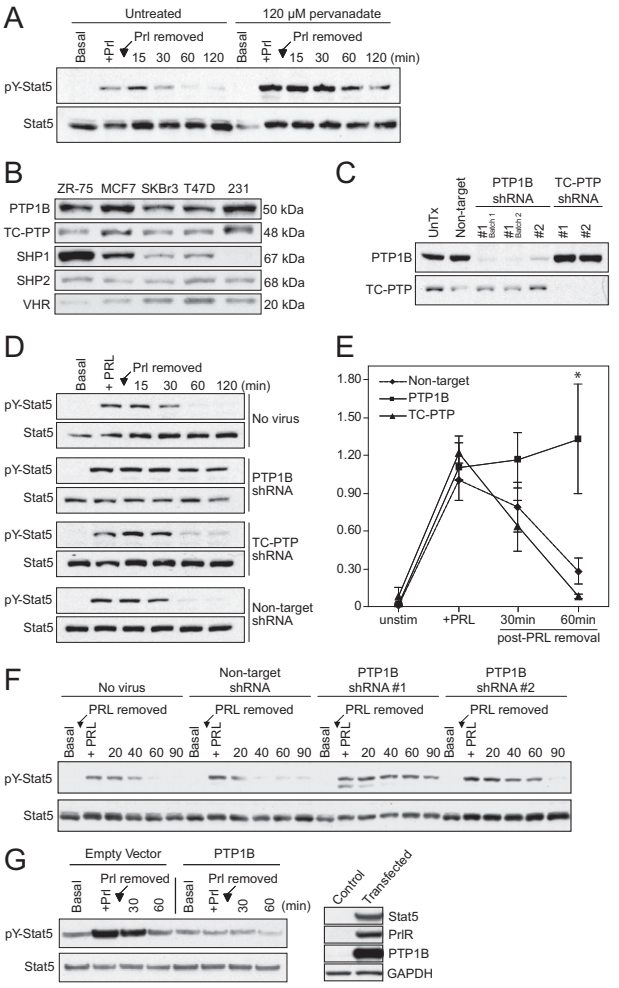


Figure 1. Phosphatase inhibition and PTP1B knockdown increased levels of phosphotyrosine-Stat5 in response to prolactin stimulation. **A:** Pervanadate inhibition of Stat5 dephosphorylation. T47D cells treated with or without 120 μmol/L pervanadate were analyzed for prolactin-induced Stat5 phosphorylation at indicated times by immunoblotting. **B:** Expression levels of candidate phosphatases in whole cell lysates from a panel of five human breast cancer cell lines. **C:** Efficacy of two lentiviral-delivered shRNA constructs on knockdown of PTP1B and TC-PTP compared to lentiviral non-target control shRNA or uninfected control T47D cells. Batch-to-batch consistency between lentiviral productions of PTP1B shRNA no.1 was also verified. **D:** Effect of PTP1B or TC-PTP knockdown on phosphotyrosine-Stat5. T47D cells were infected with lentiviral-delivered shRNA against PTP1B, TC-PTP, or a non-target control, and Stat5 phosphorylation status was measured over time following a short pulse of prolactin stimulation. **E:** Densitometry of prolactin-induced Stat5 phosphorylation following shRNA knockdown of PTP1B or TC-PTP shRNA in four independent experiments. Vertical bars indicate SEM. $^*P = 0.03$ by analysis of variance. **F:** Confirmation of prolonged Stat5 phosphorylation following PTP1B knockdown using a second shRNA construct. Prolactin-induced Stat5 tyrosine phosphorylation was measured following lentiviral infection with two independent PTP1B shRNA constructs. **G:** Effect of PTP1B overexpression on phosphotyrosine-Stat5. Prolactin receptor (PrlR), Stat5, and PTP1B were transfected into 293FT cells, and Stat5 phosphorylation was analyzed at indicated time points following prolactin pulse. **Arrows** indicate time when prolactin (Prl) was removed.

PTP1B Expression Levels but Not TC-PTP Resulted in Altered Prolactin-Induced Stat5 Tyrosine Phosphorylation

To delineate the role of the individual tyrosine phosphatases in human breast cancer cells, we used lentiviral delivery of shRNA to individually knock down each of the five candidate phosphatases. We first focused on the tyrosine phosphatases PTP1B and TC-PTP because of the key role of their common homologue, ptp61F, as a suppressor of the Jak-Stat pathway in *Drosophila*. The efficacies of five distinct shRNA constructs for PTP1B or TC-PTP were first validated (data not shown), and protein levels of PTP1B or TC-PTP were then evaluated in T47D cells using the two most effective constructs. Effective and selective knockdown of both PTP1B and TC-PTP was achieved (Figure 1C). The effect of knockdown of either PTP1B or TC-PTP on Stat5 tyrosine phosphorylation levels was then tested using the most efficacious shRNA construct. T47D cells were stimulated with a 20 minutes pulse of prolactin 72 hours after lentiviral exposure, followed by a change into prolactin-free medium. Whole cell lysates were collected at predetermined time points and immunoblotted for phosphotyrosine-Stat5 and total Stat5 protein levels. Phosphotyrosine-Stat5 levels were markedly sustained under conditions of PTP1B knockdown compared to cells treated with a non-target control shRNA or mock infection (Figure 1D). In contrast, and different from observations based on TC-PTP overexpression in Cos7 and murine mammary epithelial COMMA-1D cells,[39] TC-PTP knockdown did not affect prolactin-induced phosphotyrosine-Stat5 levels in T47D breast cancer cells (Figure 1D). Densitometric analysis of immunoblots representing four independent experiments demonstrated that Stat5 phosphorylation was significantly prolonged following PTP1B depletion, especially notable at 60 minutes post-prolactin stimulation when compared to the non-target control (analysis of variance with LSD post hoc $P = 0.03$), while TC-PTP depletion did not (Figure 1E). The effect of PTP1B knockdown to prolong prolactin-induced Stat5 phosphorylation was confirmed using a second PTP1B shRNA construct (PTP1B shRNA no. 2) (Figure 1, C and F).

To test whether PTP1B overexpression conversely would suppress prolactin-induced Stat5 phosphorylation, we transiently transfected the PrlR, PTP1B, and Stat5 into 293FT cells. Fifty-six hours after transfection the cells were serum-starved overnight and stimulated with 10 nmol/L prolactin for 15 minutes. The prolactin-containing medium was washed off, replaced with fresh serum-containing medium, and lysates were collected at predetermined time points. The lysates were immunoblotted for phosphotyrosine-Stat5, total Stat5, the PrlR, and PTP1B. PTP1B overexpression markedly suppressed prolactin-induced tyrosine phosphorylation of Stat5 (Figure 1G). Attempts to overexpress PTP1B in breast cancer cell lines T47D, MCF7, and SKBR3 resulted in undetectable or only low levels of PTP1B overexpression compared to endogenous levels and did not detectably diminish prolactin-induced Stat5 phosphorylation. Further increasing PTP1B levels in breast cancer cell lines may have limited

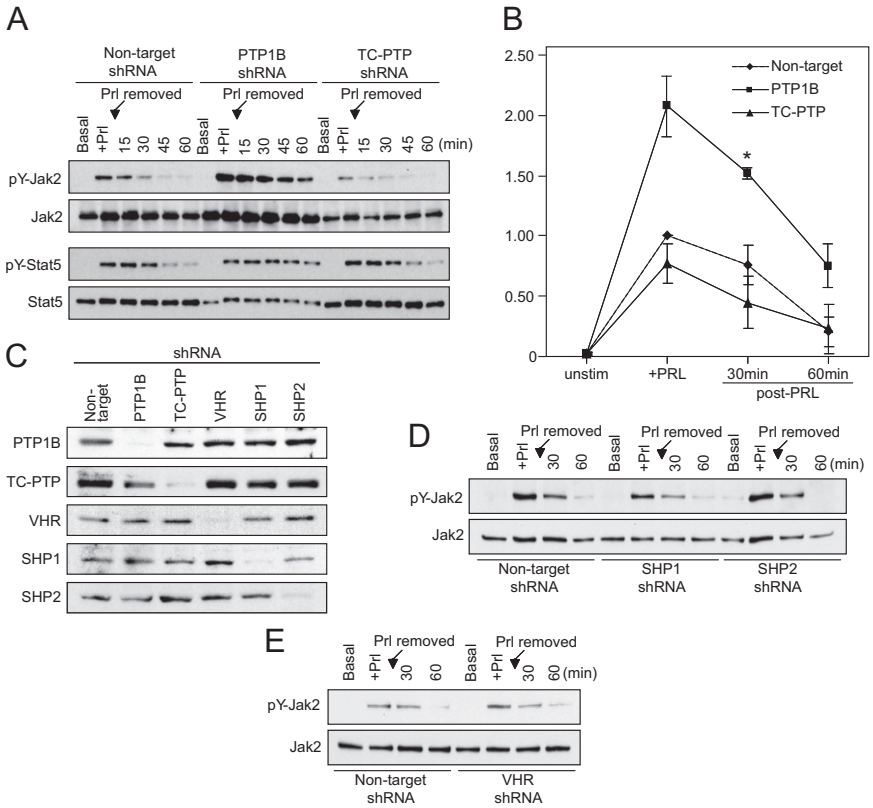


Figure 2. PTP1B knockdown, but not TC-PTP, SHP1, SHP2, or VHR, resulted in increased prolactin-induced phosphorylation of the upstream Stat5 tyrosine kinase, Jak2. **A:** Immunoblot analysis of prolactin-induced phosphotyrosine-Jak2 following knockdown of PTP1B or TC-PTP. T47D cells were infected with lentivirus expressing PTP1B or TC-PTP shRNA and stimulated for 20 minutes with 10 nmol/L prolactin, and phosphotyrosine Jak2 status was analyzed at indicated times following prolactin stimulation. **B:** Densitometry of Jak2 phosphorylation following knockdown of PTP1B or TC-PTP in three experiments. Vertical bars indicate SEM. $*P = 0.02$ by analysis of variance. **C:** Immunoblot analysis demonstrating specific phosphatase knockdown using lentiviral-delivered shRNA in T47D cells. **D:** Effect of additional phosphatases (SHP1, SHP2, and VHR) on prolactin-induced Jak2 phosphorylation. T47D cells were infected with SHP1 (**D**), SHP2 (**D**), or VHR (**E**) shRNA and analyzed by immunoblotting for prolactin-induced Jak2 phosphorylation at the indicated times following stimulation by prolactin. **Arrows** indicate time when prolactin (Prl) was removed.

efficacy due to tight regulation of already high pre-existing levels of endogenous PTP1B.

PTP1B Knockdown Modulated Tyrosine Phosphorylation of the Stat5 Tyrosine Kinase Jak2

The tyrosine kinase Jak2 has been shown to mediate prolactin-induced Stat5 phosphorylation in normal and malignant breast epithelial cells.[6,61,62] Despite previous observations in non-breast cancer cell lines that PTP1B overexpression did not affect Jak2 phosphorylation,[40] we hypothesized that the increased levels of Stat5 phosphorylation observed in response to PTP1B knockdown in T47D cells may result from a mechanism related to reduced PTP1B inhibition of Jak2, considering Jak2 is autophosphorylated within its catalytic domain on tyrosine residues 1007 and 1008, which represents a preferred substrate motif of PTP1B.[63] To address this possibility, additional time course experiments were completed in T47D cells in which the phosphorylation status of both Stat5 and Jak2 was monitored following depletion of PTP1B or TC-PTP. Cells were stimulated for 20 minutes with prolactin and whole cell lysates were collected at 15 minutes intervals for 60 minutes. In human breast cancer cells, PTP1B knockdown was associated with marked enhancement and prolongation of Jak2 tyrosine phosphorylation (Figure 2A, upper panels), consistent with prolongation of downstream Stat5 tyrosine phosphorylation (Figure 2A, lower panels), while knockdown of TC-PTP failed to alter Jak2 or Stat5 tyrosine phosphorylation (Figure 2A). Densitometric analysis of immunoblots from three experiments indicated that PTP1B knockdown significantly enhanced Jak2 phosphorylation following removal of prolactin compared to the non-target control, especially 30 minutes after prolactin withdrawal (analysis of variance with LSD post hoc $P = 0.02$), while TC-PTP

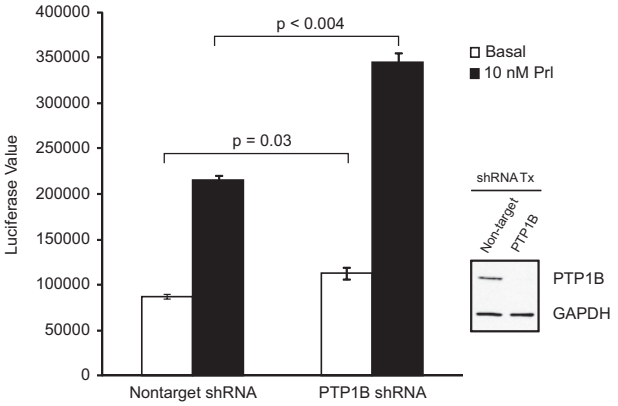


Figure 4. Stat5-responsive luciferase promoter activity was enhanced by PTP1B knockdown. T47D cells stably transfected with a Stat5-responsive β-casein luciferase reporter were treated with PTP1B or non-target control shRNA and stimulated for 8 hours with 10 nmol/L prolactin. Luciferase activity was measured and graphed as the mean of three experiments (±SD). **Inset:** Representative immunoblot confirming PTP1B knockdown.

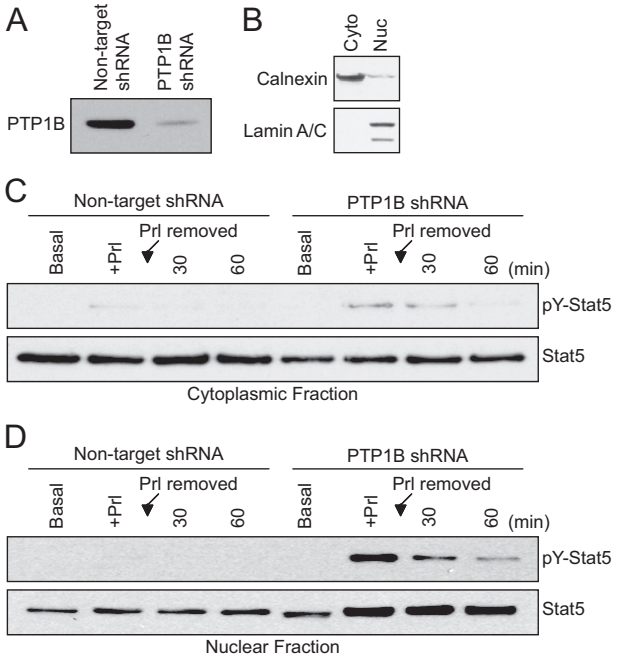


Figure 3. PTP1B knockdown enhanced prolactin-induced phosphotyrosine-Stat5 in a second breast cancer cell line, MCF7. **A:** Immunoblot confirmation of specific and effective PTP1B knockdown in MCF7 cells. **B:** Efficient fractionation of cytoplasmic and nuclear compartments from representative cell lysates using the cytoplasmic endoplasmic reticulum transmembrane protein calnexin and the nuclear proteins lamin A and C. **C** and **D:** Analysis of prolactin-induced Stat5 phosphorylation in the cytoplasmic and nuclear fractions of MCF7 cells following shRNA knockdown of PTP1B. MCF7 cells were infected with lentiviral-delivered shRNA against PTP1B or a non-target control, and fractionated lysates were evaluated over a 1-hour time course following a short pulse of prolactin for changes in phosphotyrosine-Stat5 levels. Cytoplasmic fractions (**C**) and nuclear fractions (**D**) were immunoprecipitated with anti-Stat5, immunoblotted for phosphotyrosine-Stat5, stripped, and reprobed for total Stat5 levels. **Arrows** indicate time when prolactin (Prl) was removed.

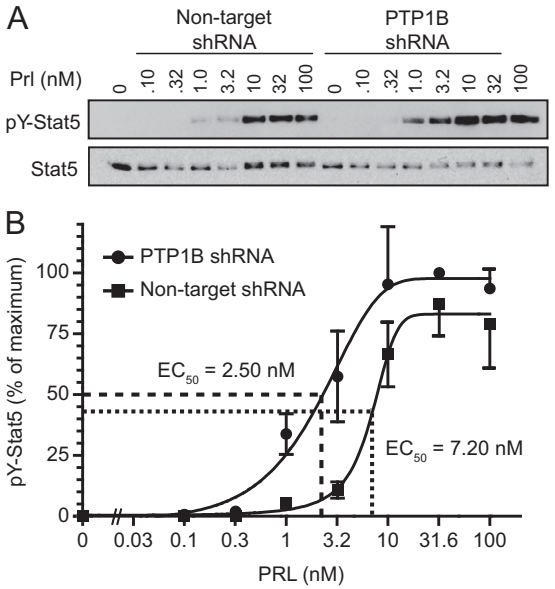


Figure 5. PTP1B knockdown resulted in hypersensitivity and hyperactivation of prolactin-induced phosphotyrosine-Stat5. T47D cells were infected with lentiviral-delivered shRNA against PTP1B or a non-target control, serum starved, and stimulated with different doses of prolactin. **A:** Whole cell lysates were collected 20 minutes following prolactin stimulation and analyzed by immunoblotting for phosphotyrosine-Stat5. **B:** Densitometry was performed from immunoblots from three experiments, and dose-response curves and EC_{50} values were calculated.

shRNA did not have this effect (Figure 2B). These observations suggest that a significant portion of the negative regulation of Stat5 tyrosine phosphorylation by PTP1B is mediated by a mechanism that involves dephosphorylation of the Stat5 tyrosine kinase, Jak2. This result is consistent with reports demonstrating that PTP1B interacts with Jak2 in signaling induced by growth hormone,[42,43] leptin,[44–47] and interferon,[48] although it is the first time such a relationship has been observed in breast cancer cells or in the setting of prolactin-Jak2-Stat5 signaling. We also established selective knockdown of SHP1, SHP2, and VHR using lentiviral delivery of shRNA (Figure 2C). However, knockdown of SHP1, SHP2, or VHR was not associated with enhanced prolactin-induced Jak2 tyrosine phosphorylation (Figure 2, D and E) or Stat5 tyrosine phosphorylation (data not shown).

PTP1B Modulation of the Prolactin/Jak2/Stat5 Pathway Also Occurs in MCF7 Breast Cancer Cells

To determine whether the role of PTP1B observed in the T47D cell line was also detectable in other breast cancer cell lines we investigated the effect of PTP1B knockdown on phosphotyrosine-Stat5 in MCF7 cells. MCF7 cells represent another luminal breast cancer cell line with relatively high levels of prolactin receptors but with generally

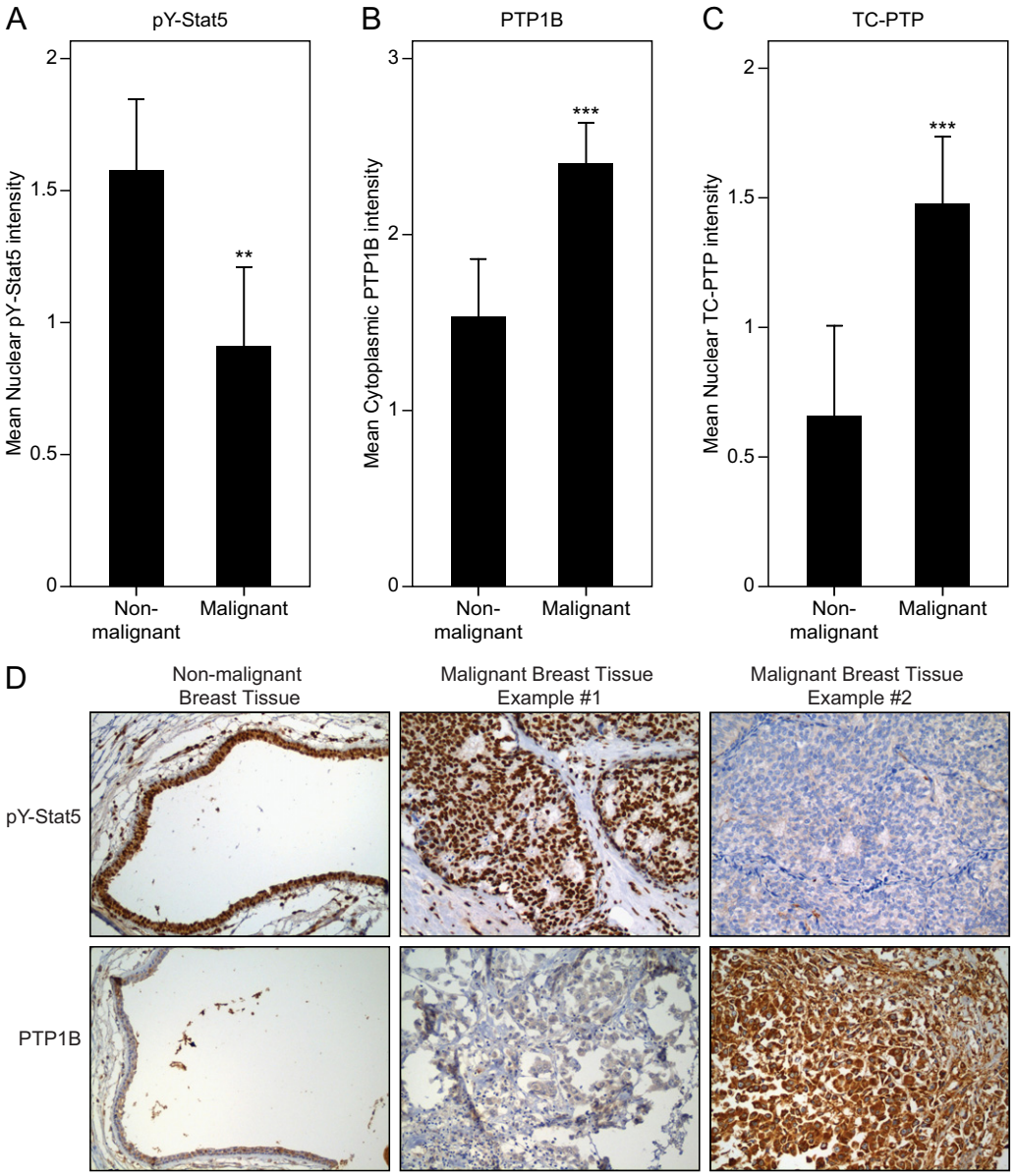


Figure 6. Expression of phosphotyrosine-Stat5, PTP1B, and TC-PTP in clinical breast tissue specimens. Phosphotyrosine-Stat5, PTP1B, and TC-PTP expression were detected by immunohistochemistry in a tissue array containing nonmalignant and malignant tissues. Expression of nuclear phosphotyrosine-Stat5 was reduced in malignant tissue compared to nonmalignant tissue (**A**) while expression of cytoplasmic PTP1B (**B**) and nuclear TC-PTP (**C**) were higher in malignant tissue. Vertical bars represent 95% confidence intervals. $^{**}P = 0.001$, $^{***}P < 0.001$ (*t*-test). **D:** Representative sections stained for phosphotyrosine-Stat5 and PTP1B of nonmalignant (example 1) and malignant (examples 2 and 3) breast.

weaker prolactin-induced Stat5 signaling than T47D cells. Successful knockdown of PTP1B protein levels was confirmed by whole cell lysate immunoblot (Figure 3A). For optimal detection of prolactin-induced Stat5 tyrosine phosphorylation, we fractionated the cytoplasmic and nuclear compartments and immunoprecipitated Stat5. Confirmation of proper cellular fractionation into nuclear and cytoplasmic components was verified by examining the levels of the nuclear pore proteins lamin A/C and the cytoplasmic endoplasmic reticulum transmembrane protein calnexin (Figure 3B). Subsequent immunoblotting was consistent with the effects seen in T47D cells, with increased and sustained Stat5 tyrosine phosphorylation in the cytoplasmic (Figure 3C) and particularly the nuclear (Figure 3D) fractions. Further confirmation of PTP1B knockdown resulting in increased Stat5 activation was provided by the observation of elevated nuclear levels of total Stat5 protein in PTP1B-depleted cells following prolactin stimulation (Figure 3D, lower panel). The observations in MCF7 cells demonstrated that the ability of PTP1B to modulate levels of Stat5 tyrosine phosphorylation was not restricted to T47D cells.

Knockdown of PTP1B Increased Levels of Stat5 Responsive β-Casein Promoter-Driven Luciferase Activity

To determine whether the increased phosphotyrosine-Stat5 levels associated with knockdown of PTP1B were functional and biologically active we used a stably transfected T47D cell line containing a Stat5-responsive β-casein promoter luciferase construct.[60] We exposed the cells to lentivirus carrying either PTP1B shRNA or non-target control shRNA, allowed 72 hours for protein knockdown to occur, and stimulated the cells for 8 hours with prolactin. The cells were then lysed, luciferase activity measured, and specific knockdown confirmed by immunoblot (Figure 4). In response to PTP1B knockdown, both basal (1.3-fold increase, $P = 0.03$) and prolactin-driven (increased from 2.5- to 4.0-fold, $P < 0.004$) luciferase activity showed statistically significant increases over the non-target shRNA control treated cells (Figure 4).

PTP1B Knockdown Increased the Prolactin Sensitivity of T47D Cells as Measured by Activation of Phosphotyrosine-Stat5 in Response to Low Doses of Prolactin

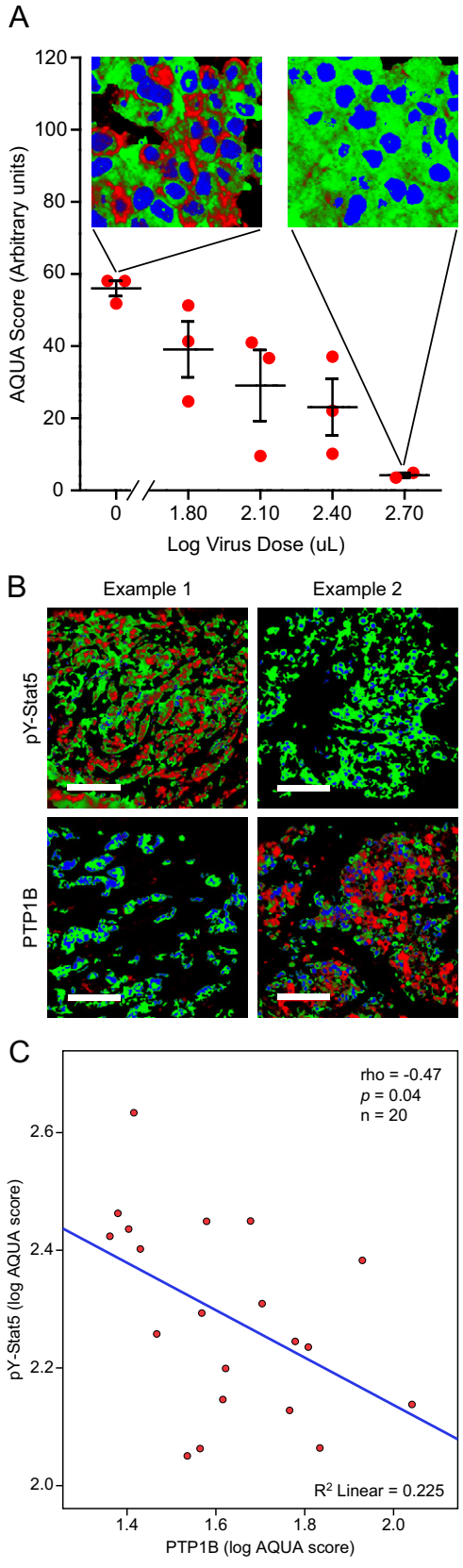
Our prior experiments demonstrated that disruption of PTP1B in T47D cells and MCF7 cells resulted in enhanced phosphotyrosine-Stat5 signaling induced by a high dose of prolactin. We therefore determined whether PTP1B up-regulation in breast cancer might represent a mechanism that reduces sensitivity of the Stat5 pathway to prolactin by investigating the effect of PTP1B knockdown on prolactin-induced Stat5 activation over a concentration range of prolactin. PTP1B-targeted shRNA-treated T47D cells were stimulated for 15 minutes with prolactin concentrations ranging from 0.1 to 100 nmol/L. Lysates were harvested and immunoblotted for active and total Stat5 (Figure 5A). Phosphotyrosine-Stat5 band intensity was quantified by densitometry from three independent experiments and dose-response curves were constructed by plotting the mean of the experiments (Figure 5B). Importantly, PTP1B knockdown resulted in a significant shift in EC_{50} from 7.20 nmol/L to 2.50 nmol/L, which represents a magnitude of approximately half of a logarithm. Also, maximum signal strength in cells treated with PTP1B shRNA was 13% greater than cells treated with control shRNA, and the response at 3.2 nmol/L prolactin was over fivefold higher in PTP1B-suppressed cells. Therefore, these results support the notion that dysregulation of PTP1B in breast cancer may significantly compromise the ability of Stat5 to act as an intracellular sensor of circulating prolactin, and provide a mechanism to explain how the increase of PTP1B activity could diminish normal phosphotyrosine-Stat5 signaling in invasive breast cancer.

Expression of the Tyrosine Phosphatase PTP1B but Not TC-PTP Is Negatively Correlated with Nuclear Localized Phosphotyrosine-Stat5 in Clinical Breast Cancer Specimens

Based on our observations in cell lines demonstrating that PTP1B serves as a negative regulator of Jak2-Stat5 activation in breast cancer, we performed immunohistochemistry of phosphotyrosine-Stat5, PTP1B, and TC-PTP in a CEMA tissue array that included 47 malignant and 48 nonmalignant human breast specimens (material I). The slides were scored blindly by an independent pathologist and the intensity of nuclear phosphotyrosine-Stat5, cytoplasmic PTP1B, and nuclear TC-PTP were recorded. While positive Stat5 status was reduced in invasive cancer (Figure 6A), PTP1B (Figure 6B) and TC-PTP (Figure 6C) expression levels were frequently increased in malignant compared to nonmalignant breast tissue (Table 1). The results of our analyses in carcinoma tissues with evaluable staining showed that the intensity of nuclear phosphotyrosine-Stat5 staining was negatively correlated with cytoplasmic PTP1B staining ($\rho = -0.38$, $P = 0.02$, $n = 37$) but not nuclear TC-PTP ($\rho = 0.11$, $P = 0.50$, $n = 39$). However, no correlation between phosphotyrosine-

Table 1. Expression of Nuclear Phosphotyrosine-Stat5, Cytoplasmic PTP1B, and Nuclear TC-PTP in Nonmalignant and Malignant Breast Tissue of Material I

	Negative	Positive	Total
Nuclear phosphotyrosine-Stat5, *n* (%)			
Nonmalignant	17 (38%)	28 (62%)	45
Malignant	33 (73%)	12 (27%)	45
Cytoplasmic PTP1B, *n* (%)			
Nonmalignant	28 (87%)	4 (13%)	32
Malignant	19 (45%)	23 (55%)	42
Nuclear TC-PTP, *n* (%)			
Nonmalignant	25 (78%)	7 (22%)	32
Malignant	25 (57%)	19 (43%)	44



Stat5 and PTP1B was seen in the nonmalignant tissues ($\rho = -0.01$, $P = 0.94$, $n = 31$) or between phosphotyrosine-Stat5 and TC-PTP ($\rho = 0.25$, $P = 0.18$, $n = 31$). Figure 6D shows representative sections of phosphotyrosine-Stat5 and PTP1B staining in nonmalignant breast tissue (upper panels) and malignant breast tissues (middle and lower panels), reflecting low PTP1B expression in tissues with high phosphotyrosine-Stat5 expression and high PTP1B expression in tissues with low phosphotyrosine-Stat5 expression. Importantly, when stratified into ER-positive and ER-negative breast cancer, the negative correlation was observed in the ER-negative population ($\rho = -0.65$, $P = 0.03$, $n = 11$) but not in the ER-positive population ($\rho = -0.22$, $P = 0.37$, $n = 19$).

To further evaluate the relationship between PTP1B and phosphotyrosine-Stat5 expression in human breast cancer, a second material (material II) was investigated using fluorescent immunostaining and quantitation by AQUA. To quantitatively validate specificity of immunostaining for PTP1B, T47D cells were treated with increasing amounts of PTP1B shRNA and analyzed for PTP1B expression in formalin-fixed, paraffin-embedded cell pellets by immunofluorescence. AQUA quantification revealed dose-dependent reduced PTP1B expression relative to amount of PTP1B shRNA (Figure 7A). AQUA was then used to correlate expression of PTP1B and phosphotyrosine-Stat5 from a second CEMA tissue array containing 100 invasive breast cancer specimens (grades 1–3). Representative immunofluorescent images of cytoplasmic PTP1B detection and nuclear phosphotyrosine-Stat5 detection from two invasive breast carcinomas are shown in Figure 7B. Consistent with the diaminobenzidine immunohistochemistry data of material I, a strong negative correlation was observed between levels of nuclear-localized phosphotyrosine-Stat5 and levels of cytoplasmic PTP1B within ER-negative invasive ductal carcinomas (Figure 7, B and C; $\rho = -0.47$, $P = 0.04$, $n = 20$) and not in ER-positive cases ($\rho = 0.15$, $P = 0.43$, $n = 30$).

Discussion

The present study is the first to implicate tyrosine phosphatase PTP1B as a major negative modulator of prolactin-stimulated Stat5 tyrosine phosphorylation in human breast cancer cell lines based on several lines of evidence. First, we observed prolonged elevation tyrosine phosphorylated Stat5 in prolactin-responsive breast cancer cell lines following knockdown of PTB1B using two independent shRNAs but not following knockdown of other candidate phosphatases that have been implicated

Figure 7. PTP1B expression increases with histological grade and is inversely correlated with nuclear phosphotyrosine Stat5 expression. PTP1B and phosphotyrosine-Stat5 expression were analyzed by immunofluorescent staining and quantified by AQUA on a CEMA tissue array representing 100 invasive breast carcinoma specimens (grades 1–3). **A:** Validation of PTP1B immunofluorescent staining and detection by AQUA demonstrated a dynamic range of PTP1B protein expression levels in T47D cells treated with increasing amounts of PTP1B shRNA. **B:** Representative sections of the CEMA tissue microarray stained for phosphotyrosine-Stat5 and PTP1B. **C:** Scatter plot of phosphotyrosine-Stat5 intensity and PTP1B intensity in ER-negative invasive ductal carcinoma patients.

in Stat5 modulation in other cell types, including TC-PTP modulation of prolactin-induced Stat5 phosphorylation in Cos7 and COMMA-1D murine mammary epithelial cells.[39] Conversely, overexpression of PTP1B diminished prolactin-induced Stat5 tyrosine phosphorylation. Second, there was hyperresponsiveness and increased sensitivity of Stat5 to extracellular prolactin stimulation in T47D cells deficient in PTP1B, providing a mechanism to explain how up-regulation of PTP1B frequently observed in breast cancer could compromise Stat5 activation in breast cancer. Last, a negative correlation was detected between expression of PTP1B and levels of nuclear localized, tyrosine phosphorylated Stat5 in two independent clinical breast cancer materials. Furthermore, we determined based on PTP1B gene knockdown in T47D human breast cancer cells that PTP1B negatively regulates phosphorylation of the upstream Stat5 kinase, Jak2.

The stimulatory effect of PTP1B knockdown on prolactin-induced tyrosine phosphorylation of Stat5 in breast cancer cells was matched by a comparable effect on levels of tyrosine phosphorylated Jak2, the upstream Stat5 tyrosine kinase. Based on this observation, a majority of the suppressive effect of PTP1B on Stat5 tyrosine phosphorylation in breast cancer cells may involve PTP1B-mediated inactivation of Jak2, an observation consistent with a growing body of related evidence from studies of cytokine receptor signaling in non-mammary model systems such as the growth hormone,[42,43] leptin,[44–47] and interferon[48] pathways, but unexpected in light of previous observations of prolactin-mediated Jak2-Stat5 signaling in non-breast cancer cells.[40] In addition, PTP1B has a 70-fold preference for tandem phosphotyrosines in the motif (E/D)-phosphotyrosine-phosphotyrosine-(R/K), a sequence found in the activation loop of Jak2, over mono-phosphotyrosine sites such as the activating tyrosine on Stat5.[63] While we cannot rule out that PTP1B may also directly dephosphorylate Stat5 in breast cells, our evidence best supports a model of PTP1B regulating prolactin-activated Jak2 directly with the effects on phosphotyrosine-Stat5 secondary to modulation of Jak2 kinase activity. Importantly, the present report implicates PTP1B as a regulator of prolactin-mediated Jak2 and Stat5 phosphorylation in human breast cancer, providing a mechanism to explain the observed loss of Stat5 activation in a significant portion of progressing breast cancer.[8]

PTP1B has been reported to be up-regulated in human breast cancer, a finding that correlated with aggressive morphological characteristics,[64] but until now no connection had been made between PTP1B and compromised Stat5 activation. Interestingly, the negative correlation between levels of PTP1B and tyrosine phosphorylated Stat5 was most consistent in ER-negative breast cancer. Furthermore, PTP1B is a known mediator of downstream HER2 signaling via Src and ERK,[65,66] and PTP1B expression is increased in cells lines that were transformed by HER2,[67,68] raising the possibility that PTP1B could also serve as a mediator for inhibitory cross talk between the HER2 and Stat5 pathway. However, such a relationship remains to be investigated in larger materials. Studies of spontaneous mammary carcinomas in mice have demonstrated a potentially critical role for PTP1B in metastatic progression. In the NDL2 mouse model, genetic loss or pharmacological inhibition of PTP1B decreased lung metastases.[69] A potential function of PTP1B in breast cancer progression was recently suggested based on observations that PTP1B modulated c-Src activity and further implicated PTP1B in the regulation of invadopodia formation and breast cancer invasion.[70] Although PTP1B may regulate breast cancer invasiveness by multiple mechanisms, inhibition of prodifferentiative and invasion-suppressive effects of the Stat5 pathway[12] could be an important factor not previously considered. A separate study showed homozygous deletion of PTP1B in another HER2-driven system, the MMTV-*NeuNT* mouse, delayed or prevented mammary tumorigenesis altogether.[71] However, analysis of several important signaling molecules in the MMTV-*NeuNT* model identified decreased Erk activation in the preneoplastic glands as a possible contributor to the PTP1B phenotype but noted no changes in the phosphorylation of AKT, SRC, FAK, or Stat5 in either preneoplastic glands or tumor lysates.

This report investigated whether disruption of normal phosphatase expression could provide a mechanism to explain how phosphotyrosine-Stat5 is lost during breast cancer progression. We described how knockdown of PTP1B resulted in increased levels of phosphotyrosine-Jak2, the upstream activating kinase of Stat5, and subsequently phosphotyrosine-Stat5 itself. Furthermore, inhibition of normal PTP1B expression resulted in hypersensitive and hyperactive Stat5 in response to low levels of prolactin stimulation providing a mechanistic link between expression levels of a regulatory tyrosine phosphatase and signal transduction through the prolactin/Jak2/Stat5 pathway. Ultimately, our biochemical, biological, and mechanistic data were consistent with immunohistochemical analyses of clinical breast cancer specimens, which revealed a significant negative correlation between PTP1B expression and the level of nuclear phosphotyrosine-Stat5. In summary, up-regulation of PTP1B activity could represent an important mechanism behind the frequently diminished Stat5 activation in breast cancer. While other mechanisms may also be responsible for decreased Stat5 tyrosine phosphorylation, phosphatases represent intriguing druggable targets that could lead to new strategies for breast cancer therapy.

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