Protein-Tyrosine Phosphatase H1 Increases Breast Cancer Sensitivity to Antiestrogens by Dephosphorylating Estrogen Receptor at Tyr537

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Abstract

Estrogen receptor α (ERα or ER) is the only target of breast cancer therapy using antiestrogens. However, about 50% of ER-expressing breast cancer is intrinsically refractory to the antihormone therapy and strategies to improve the therapeutic response are urgently needed. Dynamic ER phosphorylation and dephosphorylation play an important role in ER activity and antihormone response. Although more than 10 kinases participate in phosphorylating ER protein, phosphatases involved remain mostly unidentified. Here, we tested the hypothesis that the protein-tyrosine phosphatase H1 (PTPH1) may regulate ER tyrosine phosphorylation and thereby impact breast cancer antihormone sensitivity. Our results showed that PTPH1 dephosphorylates ER at Tyr537 in vitro and in breast cancer cells. Moreover, PTPH1 stimulates ER nuclear accumulation and increases breast cancer sensitivity to tamoxifen (TAM) and/or fulvestrant in cell culture and in a xenograft model. Further analysis revealed that PTPH1 depends on its catalytic activity to stimulate ER nuclear accumulation and to enhance breast cancer antihormone sensitivity. These studies thus identified PTPH1 as a novel ER phosphatase and further demonstrate a therapeutic potential of enhancing breast cancer sensitivity to antiestrogens through dephosphorylating ER by PTPH1.

Introduction

Estrogen receptor α (ERα or ER) is expressed in about 70% of breast cancer and is activated by estrogen and growth factors to regulate expression of genes important for breast cancer growth (1). Consequently, ER serves as a key target for antihormone therapy by using antiestrogens such as tamoxifen (TAM) and fulvestrant (2). However, about 50% of ER-positive (ER+) breast cancers are refractory to the antihormone therapy and strategies to improve the therapeutic response are urgently needed (2, 3). ER is an important nuclear receptor and phosphorylation plays an important role in determining ER activity and hormone response by a residue-specific mechanism (2, 4). In response to estrogen and growth factors, for example, ER is phosphorylated at S118 by ERK (extracellular signal-regulated kinases; ref. 5), Cdk7 (6), and p38 MAPK (mitogen-activated protein kinase; ref. 7). Increased levels of p-ER/S118 results in enhanced breast cancer sensitivity to TAM (7) and hyperexpressed p-ER/S118 in clinical breast cancer further correlates with a better response to the TAM therapy (8). ER phosphorylation at S305 by PAK1 (p21-activated kinase 1), on the other hand, reduces cellular response to TAM (9), and a cooverexpression of PAK1 with p-ER/S305 in clinical breast cancer is associated with antihormone resistance (10). Studies of ER phosphorylation may therefore contribute to the development of novel strategies to improve the clinical efficacy of antihormone therapy.

Tyrosine 537 (Tyr537 or Y537) phosphorylation of ER by Src plays an important role in ER dimerization and DNA-binding activity (11, 12). Recent studies further showed that Src-induced ER/Y537 phosphorylation is essential for ER nuclear export (13) and for E6AP-triggered ER degradation (14). Furthermore, increased levels of p-ER/Y537 expression in clinical breast cancer are associated with a poor therapeutic response to TAM (15) and the Tyr537Asn mutation was detected in metastatic breast cancer (16). These results together indicate an important role of Y537 in ER activity and antihormone response. Although several studies have demonstrated ER/Y537 phosphorylation by Src family kinases (11, 12, 17), phosphatases involved in ER/Y537 dephosphorylation are completely unknown.

Protein-tyrosine phosphatase H1 (PTPH1, also called PTPN3) is a 120-kDa protein that belongs to the nontransmembrane PTP superfamily (18). Previous genetic analysis showed that PTPH1 and its several family members are mutated in human colon cancer but the functional
consequence of these mutations remains unestablished (19). Our recent studies showed that PTPH1 dephosphorylates and cooperates with p38 MAPK to promote Ras oncogenesis through a complex formation (20, 21). Importantly, PTPH1 is overexpressed in breast cancer and stimulates breast cancer growth in vitro through collaboration with vitamin D receptor (22). In this report, we tested the hypothesis that PTPH1 may dephosphorylate ER at Tyr537 and thereby regulate breast cancer antihormone sensitivity. Our results showed that PTPH1 induces ER/Y537 dephosphorylation in vitro and in vivo, and thereby increases breast cancer sensitivity to TAM and fulvestrant in cell culture and in a mouse xenograft model. Targeting ER/Y537 dephosphorylation may therefore be a novel approach to improve breast cancer sensitivity to antiestrogens.

Materials and Methods

**Plasmids, constructs, cell lines, and reagents**

MCF-7, T47D, ZR-75-1, and 293T cells were obtained from American Type Culture Collection and are maintained free of contamination (7, 22), but no authentication was done by the authors. Plasmids GFP-ERα and their Y537F mutant were described previously (13). HA-tagged wild-type (WT) PTPH1 and its phosphatase-deficient mutant PTPH1/DA were kindly provided Dr. N.K. Tonks (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; refs. 18, 23) and used previously in our laboratory (20-22). The pLent6i/Block-it system (Block-itTM U6 RNAi entry vector Kit, Cat: K4944-00 and Block-itTM Lentiviral RNAi expression system, Cat: K4943-00; Invitrogen) was used to clone sequences for short hairpin RNAs (shRNA). The details of the target sequences used for luciferase and PTPH1 (shLuc and shPTPH1) are described earlier (20–22). Antibody against PTPH1 (mouse) was kindly provided by Dr. N.K. Tonks and other antibodies used in this study include anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase; sc-47724), anti-ERα (sc-543), anti-p-ERα/Y537 (sc-32827), anti-ERα (sc-8002), anti-phospho-Tyr (sc-18182 and sc-508), anti-c-jun (sc-44), anti-GFP (sc-9996), anti-α-actinin (sc-17829), and anti-α-tubulin (sc-6199) were obtained from Santa Cruz Biotechnology. Cycloheximide (CHX), estradiol (E2), and 4-hydroxy-TAM were purchased from Selleckchem. 

**Gene expression and silencing**

PTPH1 and PTPH1/DA stably expressed T47D cells were generated as described earlier (22). A tetracycline-inducible (Tet-on) system (Invitrogen) was used to express PTPH1 in MCF-7 cells (7, 22, 24). These cells were typically incubated with and without Tet for 24 hours to induce PTPH1 expression, which were then used for various experiments. Silencing of PTPH1 in MCF-7, T47D, and ZR-75-1 cells was achieved by infections with lentiviruses containing shPTPH1 or control shLuc, followed by antibiotic selection as described previously (22).

**Cell fractionation, immunoprecipitation, and immunoblot analysis**

MCF-7 and T47D cells were subjected to cell fractionation following overexpression or silencing of PTPH1 under different experimental conditions. The protocol was followed as described earlier (22). Briefly, cells were lysed in a buffer containing 20 mmol/L HEPES, pH 7.4, 5 mmol/L KCl, 137 mmol/L NaCl, 5.5 mmol/L glucose, 10 μmol/L EDTA, 0.05% Nonidet P-40, 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL leupeptin, and 1 μg/mL aprotinin, followed by incubation at 4°C for 20 minutes. Thereafter, lysates were subjected to centrifugation at 1,000 rpm to separate cytoplasmic protein from nuclei. The nuclear pellets obtained were washed and resuspended in 200 μL of buffer containing 1% Triton X-100, 400 mmol/L KCl, 10 mmol/L Tris-HCl, pH 7.5, 0.1 mmol/L PMSF, 1 μg/mL aprotinin, and 1 μg/mL leupeptin.

For immunoprecipitation analyses, cells were washed with cold PBS and lysed in modified RIPA buffer (50 mmol/L Tris-HCl, pH 7.5, 1 mmol/L PMSF, 1 mmol/L dithiothreitol, 10 mmol/L sodium fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 μg/mL peptatin containing 1% Nonidet P-40) and used previously (22). Proteins were subjected to immunoprecipitation with different antibodies, including anti-GFP (sc-9996), anti-p-ERα/Y537 (sc-32827). An aliquot containing 10% of the total lysates was used as an input control. Thereafter, immunoprecipitates were washed and analyzed by Western blot analysis as previously described (22).

**Colony formation, TAM treatment with antiestrogens, and ER stability studies**

For colony formation, breast cancer cells (MCF-7, T47D, and ZR-75-1) engineered to overexpress or silence PTPH1 were plated (500–1,000) in 6-well plates and treated with TAM or fulvestrant at different concentrations as indicated. Colony formation was stained, photographed, and manually counted about 2 weeks later following the protocol as reported earlier (22). To assess ER protein stability, 293T or MCF-7 cells (Tet-on PTPH1) were transiently transfected with indicated plasmids in the experiment. Cells were then treated with CHX (100 μg/mL) for the time as indicated and lysates were collected for Western blot analysis.

**ER/Y537 dephosphorylation in vitro and in cells**

Glutathione S-transferase (GST) and GST-PTPH1 proteins were purified from IPTG (isopropyl-1-thio-B-[scap] d[r]-galactopyranoside)–induced BL21 (DE3) cells using glutathione beads. The protocol for purification of proteins and in vitro dephosphorylation assay is described earlier (20). In vitro dephosphorylation of ER/Y537 was carried out as previously described (20). Briefly, transiently expressed GFP-ER in 293T cells was isolated with anti-GFP antibody to purify the exogenous ER, whereas endogenous ER in MCF-7 cells was isolated by anti-p-ER/Y537 to collect the phosphorylated ER proteins.
Purified proteins were then incubated with GST and GST-PTPH1 in vitro for phosphatase activity assays (20). Phosphorylated proteins were then separated by an SDS-PAGE and detected with either an anti-p-Tyr. For ER/Y537 dephosphorylation in cells, MCF-7 cells were cultured for 24 hours in medium without serum and phenol red, which were then pulsed with estrogen (100 nmol/L for 30 minutes), and lysates were then subjected to immunoprecipitation with p-ER/Y537 antibody. Precipitates were subjected to Western blot analysis with ER or p-Tyr antibody (14).

**Breast cancer xenograft studies**

Animal experiments were conducted in accordance with the approved protocols of the Medical College of Wisconsin Institutional Animal Care and Use Committee. Briefly, vector- (LacZ) or PTPH1-transfected T47D cells were collected from cell culture, washed with cold PBS, and suspended in Matrigel (BD Biosciences) at 50% (v/v in PBS). Cells (5 \times 10^6) in 50 μL volume were subcutaneously injected into athymic female nude mouse (Charles River Laboratories) at both fronts. To support the growth, PBS was then injected into the tumor in equal volume 3 days after tumor cell injection. To induce tamoxifen (TAM) treatment, mice were injected subcutaneously with 2.5 μg of β-estradiol 17-valerate in 100 μL of peanut oil once per week as described before (25). When tumors have reached approximately 30 to 60 mm³ (day 9 after the inoculation), animals were randomly divided into the therapy group (TAM citrate, 500 mg/g body weight), control group (the same volume of peanut oil), and normal group (normal diet and water). For TAM treatment, TAM (500 mg/g body weight) was administered to the animals orally. The dosage of TAM was chosen as reported earlier (26). Tumor growth was monitored up to 18 days after TAM therapy. Tumor was measured twice per week using a caliper and the volume was calculated with a formula, \( V = \frac{a \times b \times c}{6} \), where a, b, and c represent tumor length, width, and height, respectively; ref. 27). To confirm the PTPH1 overexpression in tumors, lysates were prepared at the end of experiment for Western blot analysis (20).

**Statistical analysis**

Colony numbers and tumor volume were analyzed by the Student t test for statistical difference and the P value less than 0.05 is considered as statistically significant.

**Results**

**PTPH1 dephosphorylates ER/Y537 in vivo and in vitro**

To examine whether PTPH1 dephosphorylates ER/Y537 in breast cancer cells, PTPH1 was expressed in MCF-7 cells by a tetracycline inducible system (Tet-on; ref. 22). After 24 hours incubation with and without Tet in serum starvation condition, cells were pulse-treated with estrogen (E2) for 30 minutes and phosphorylated ER/Y537 proteins were purified by a specific antibody (p-ER/Y537). Precipitates were then analyzed by Western blot analysis with an ER antibody (14). Results in Fig. 1A showed that estrogen treatment increased levels of p-ER/Y537, which, however, were significantly decreased by Tet inducible PTPH1 expression with and without estrogen treatment. Because the p-ER/Y537 antibody cannot recognize p-ER/Y537 in direct Western blot analysis (14), we assessed total ER and PTPH1, p-ER/Y537, and GAPDH levels of p-ER/Y537, which were significantly decreased by Tet inducible PTPH1 expression with and without estrogen treatment. Because the p-ER/Y537 antibody cannot recognize p-ER/Y537 in direct Western blot analysis, we assessed total ER and PTPH1, p-ER/Y537, and GAPDH levels of p-ER/Y537, which were significantly decreased by Tet inducible PTPH1 expression with and without estrogen treatment. Because the p-ER/Y537 antibody cannot recognize p-ER/Y537 in direct Western blot analysis (14), we assessed total ER and PTPH1, instead of p-ER/Y537, protein expression in whole-cell lysates (WCL), which showed that PTPH1 is overexpressed after Tet addition (Fig. 1A, input). These results together demonstrated that inducible PTPH1 overexpression dephosphorylates ER/Y537 in breast cancer cells. To determine whether PTPH1 directly catalyzes ER tyrosine dephosphorylation, GFP-tagged ER proteins were transiently expressed in 293T cells. The same amount of GFP precipitates was incubated with GST or GST-PTPH1 in vitro for phosphatase activity assay as previously described (20), followed by WB analysis using a p-Tyr–specific antibody with the IgG band as a control (right).

**Figure 1.** PTPH1 dephosphorylates ER/Y537. A, PTPH1 dephosphorylates ER/Y537 in MCF-7 cells. Tet-on PTPH1 MCF-7 cells were grown in serum-free and phenol red–free medium for 24 hours in the presence and absence of Tet, and then treated with estrogen (E2, 100 nmol/L) for 30 minutes. Phosphorylated ER/Y537 proteins were isolated (all the same as described in B). Phosphorylated proteins were then separated by an SDS–PAGE and detected with either an p-Tyr antibody. The same membrane was stripped and reprobed with a phospho-specific antibody and precipitates were analyzed by Western blot analysis (14), with an IgG band as a control. A portion of lysates was also analyzed by direct WB analysis as an input. B, PTPH1 dephosphorylates ER/Y537 in vitro. GFP-ER was transiently expressed in 293T cells for 48 hours and the same amount of GFP precipitates were incubated with GST or GST-PTPH1 for phosphatase activity assay as previously described (20), followed by WB analysis using a p-Tyr–specific antibody with the IgG band as a control (right).
residue-specific phospho antibody (Fig. 1A), precipitates were incubated with GST or GST-PTPH1 in vitro, and levels of p-ER/Y537 proteins were then detected with a specific p-Tyr antibody. Results in Fig. 1B (right) showed that GST-PTPH1 again decreases levels of the tyrosine-phosphorylated ER. These results together demonstrated that PTPH1 catalyzes ER/Y537 dephosphorylation in vitro and in breast cancer cells.

PTPH1 increases breast cancer sensitivity to TAM and/or fulvestrant in vitro and in a mouse xenograft model

We previously reported that PTPH1 is overexpressed in clinical breast cancer (22). Because higher levels of p-ER/Y537 protein expression are associated with a poorer clinical response to TAM therapy (15), we next investigated whether PTPH1 may increase breast cancer sensitivity to TAM through decreasing ER phosphorylation at Y537. In this regard, two ER+ breast cancer cell lines were overexpressed with PTPH1 by a stable transfection (T47D cells) or Tet-on system (MCF-7 cells; ref. 22). Resultant cells were cultured with and without TAM for about 2 weeks for their colony forming activities as previously described (22). Results in Fig. 2A and B (and Supplementary Fig. S1A and S1B) showed that PTPH1 overexpression in both cell lines significantly increases the growth inhibition by TAM as compared with their respective controls. A similar sensitization was also observed to fulvestrant,

![Figure 2. PTPH1 affects breast cancer cell sensitivity to TAM and fulvestrant. A and B, PTPH1 overexpression increases growth inhibition by TAM. PTPH1 was overexpressed by a Tet-on system or a stable transfection, and resultant cells were incubated with TAM as indicated for about 2 weeks. Colony formed was stained and counted. Results shown are normalized to its own solvent control of Vector and PTPH1-overexpressed cells, respectively (means ± SD; n = 3–5) with insets showing PTPH1 overexpression. C and D, PTPH1 depletion causes the resistance to TAM-induced growth inhibition. PTPH1 was depleted by lentiviral-mediated shRNA delivery, and resultant cells were assessed for TAM-induced growth inhibition by colony formation as described above. Results shown are normalized to its own solvent control of shLuc and shPTPH1 cells, respectively (means ± SD; n = 3–5, with insets showing PTPH1 depletion). E and F, PTPH1 overexpression increases the growth inhibition by fulvestrant. Experiments were conducted as described in Fig. 2A and B and results are from three experiments (means ± SD). * versus vector or no Tet cells for A and B, versus shPTPH1 cells for C and D, and versus vector or no Tet cells for E and F.]
another antiestrogen (Fig. 2E and F and Supplementary Fig. S2A; ref. 28). Moreover, PTPH1 overexpression in T47D cells also increases cell death induced by higher concentrations of TAM (Supplementary Fig. S2B). In addition, PTPH1 knockdown by lentiviral-mediated shRNA delivery (22) attenuates the TAM growth inhibitory activity in both lines (Fig. 2C and D and Supplementary Fig S1C and S1D). A similar effect was also observed in ZR-75 cells following the PTPH1 depletion (Supplementary Fig. S1E). These results together demonstrated that cellular levels of PTPH1 protein expression positively determine breast cancer sensitivity to TAM and fulvestrant.

To show whether PTPH1 increases the TAM sensitivity in a breast cancer xenograft, T47D cells stably transfected with PTPH1 (and vector) were subcutaneously injected into Balb/c female nude mice and their tumor-forming activity was examined with and without the systemic TAM therapy. Consistent with the growth-stimulatory activity of PTPH1 in vitro (22), the PTPH1 expression in T47D cells significantly increases the tumor growth as compared with tumors formed by the same number of vector-transfected cells before TAM therapy at both time points after inoculation (Fig. 3A). An overall growth pattern of the xenografts formed by vector-transfected T47D cells in solvent treated group (Fig. 3B) is similar to those reported before (29, 30). Therapy with TAM (26) decreases the tumor growth both in vector and PTPH1 groups (Fig. 3B). However, a significant growth inhibition by TAM was observed day 3 after the therapy in PTPH1 tumor but day 7 in vector (LacZ) group (Fig. 3B and C). Moreover, the sustained and significant growth inhibition by TAM in PTPH1 tumor was observed throughout the experiment (*, P < 0.05 for all time points beginning day 3 after TAM vs. solvent control; Fig. 3B and C). Western blot analysis of tumor lysates confirmed increased PTPH1 expression in T47D/PTPH1 tumors with and without TAM therapy (Fig. 3C, inset). These results together demonstrated that the PTPH1 forced expression increases the growth inhibitory activity of TAM in a breast cancer xenograft model.

PTPH1 increases ER nuclear accumulation and enhances ER degradation via Y537

ER/Y537 is required for estrogen-induced ER nuclear export (13), whereas a translocation of ER out of the nucleus is associated with TAM resistance (31). We therefore next examined whether PTPH1 regulates ER cellular localizations, which may contribute to its antihormone sensitization effect. Cell fractionation and Western blot analysis showed that Tet inducible PTPH1 overexpression in MCF-7 cells does not significantly impact total ER protein expression in WCLs, but significantly increases nuclear ER accumulation (Fig. 4A). The enhancing effect of PTPH1 on ER nuclear accumulation was further
demonstrated in PTPH1 stably expressed T47D cells, which seems to be unaffected by E2 addition, albeit less significant under this condition (Fig. 4B). Moreover, PTPH1 depletion has an opposite effect on nuclear ER protein levels in both lines (Fig. 4C and D). Although PTPH1 silencing increases total ER protein levels in MCF-7 cells, this effect is not reproducible (data not shown) and was not observed in T47D cells (Fig. 4C and D), likely as a result of a different cell proliferative status as previously observed (32). These results together demonstrated that PTPH1 stimulates ER nuclear accumulation independent of ligand in breast cancer cells. One recent study showed that Y537 is required for estrogen-induced ER degradation by proteasomal pathways (14). We next assessed whether Y537 is involved in intrinsic ER protein stability with and without PTPH1 overexpression. In this case, GFP-tagged WT ER and its ER/Y537F mutant were transiently expressed in Tet-on PTPH1 MCF-7 cells. After incubation with and without Tet, cells were cultured with CHX, a protein synthesis inhibitor, and levels of ER/PTPH1 protein expression were examined by Western blot analysis as described previously (24). Results in Fig. 5A showed that ER/Y537F is degraded more rapidly than WT ER protein. This result is different than ligand-induced ER degradation in 231 breast cancer cells (14), which may represent a cell line–specific and/or ligand-dependent effect. However, Tet-induced PTPH1 expression increases the degradation of WT but not its Y537F mutant, whereas GFP-ER or GFP-ER/Y537 expression has no substantial effect on PTPH1 protein expression (Fig. 5A). These results indicate that Y537 is required for intrinsic ER protein stability and PTPH1 enhances ER degradation.
protein turnover through Y537, whereas both ER and ER/Y537 expression have no significant effects on PTPH1 protein stability. The same conclusion was also reached in 293T cells by transient cotransfections of PTPH1 with ER or ER/Y537F, albeit expressed ER seemed to have a mild effect to enhance the cotransfected PTPH1 degradation as compared with the ER/Y537F (Fig. 5B). These results together indicate that Y537 is required for intrinsic ER protein stability and PTPH1 depends on Y537 to increase ER protein turnover, which may play a role in PTPH1 stimulating ER nuclear accumulation and increasing breast cancer sensitivity to antiestrogens.

**PTPH1 depends on its catalytic activity to increase ER nuclear accumulation and to enhance breast cancer antihormone sensitivity**

Thus far, we have shown that PTPH1 dephosphorylates ER/Y537, stimulates ER nuclear accumulation, and increases breast cancer sensitivity to TAM and fulvestrant. To examine whether PTPH1 causes these effects through its phosphatase activity, its catalytic inactive mutant PTPH1/DA (23) was stably expressed in T47D cells. Resultant effects on ER localizations, p-ER/Y537 expression, and the growth inhibition by TAM were analyzed as compared with cells transfected with WT PTPH1. Results in Fig. 6A showed that the DA expression has a much less effect on increasing nuclear ER accumulation as compared with PTPH1-expressed cells. Western blot analysis of p-ER/Y537 precipitates from PTPH1/DA stably expressed cells showed no decreased but rather increased p-ER/Y537 expression as compared with the vector control (Fig. 6B), likely as a result of an increased substrate binding activity of this trapping mutant (33). Most importantly, there are no substantial differences in TAM-induced growth inhibition by the PTPH1/DA expression as compared with the vector control, whereas the PTPH1 expression significantly increases the TAM sensitivity (Fig. 6C and Supplementary Fig. S1F). These results together indicate that PTPH1 depends on its phosphatase activity to increase ER nuclear accumulation and to enhance breast cancer antihormone sensitivity.

**Discussion**

ER phosphorylation plays a determinant role in breast cancer antihormone sensitivity (2, 4). Our results presented here have advanced this field by demonstrating that ER dephosphorylation at a critical residue such as Y537 is equally important for breast cancer response to antihormone therapy through increasing ER nuclear accumulation. First, we showed that PTPH1 dephosphorylates ER/Y537 in vitro and in breast cancer cells thus acting as a novel ER phosphatase (Fig. 1). Furthermore, PTPH1 was...
demonstrated to enhance ER protein degradation and to increase ER nuclear accumulation depending on Y537 (Figs. 4 and 5). These results indicate a role of PTPH1-induced and Y537-dependent ER protein turnover that may be involved in breast cancer antihormone sensitivity through nuclear/cytoplasmic shuttling. Most importantly, PTPH1 increases breast cancer antihormone sensitivity in cell culture and in a mouse xenograft model, and requires its catalytic activity and/or Y537 to increase ER nuclear accumulation and ER turnover, and/or to enhance the TAM-induced growth inhibition (Figs. 3–6). These results together indicate that it is PTPH1-induced ER/Y537 dephosphorylation that integrates the ER nuclear accumulation/turnover with the enhanced antihormone sensitivity. Because increased p-ER/Y537 expression in clinical breast cancer correlates with TAM resistance (15), our results provide the critical experimental evidence to indicate an application potential of decreasing p-ER/Y537 expression by PTPH1 in increasing breast cancer antihormone sensitivity. This possibility is further supported by the fact that PTPH1 is overexpressed in about 50% of clinical breast cancer (22). Targeting ER/Y537 dephosphorylation by PTPH1 in breast cancer may therefore be a novel approach to improve the clinical response to antiestrogens.

PTPH1 may increase breast cancer antihormone sensitivity by stimulating nuclear ER accumulation through dephosphorylating p-ER/Y537 independent of ER transcriptional activity. This is because PTPH1 does not affect ER transcriptional activity in breast cancer cells (22) but depends on its phosphatase activity to increase ER nuclear accumulation and to enhance TAM-induced growth inhibition (Fig. 6). Moreover, ER/Y537 is required for ER nuclear export and its nonphosphorylable ER/Y537m mutant remains mostly nuclear independent of estrogen (13), an effect similar to that observed with endogenous ER protein in response to PTPH1 overexpression (Fig. 4). In addition, consistent with our findings of PTPH1 increasing breast cancer antihormone sensitivity through stimulating ER nuclear accumulation, previous studies showed that increased cytoplasmic ER contents and/or activated nongenomic ER signaling can lead to resistance to TAM (26, 31). However, we cannot rule out whether Y537-independent mechanisms may also be involved in the PTPH1-induced sensitization. One recent study reported that ER is also phosphorylated at Y52 and/or Y219 by c-Abl, which is involved in breast cancer invasion and growth (34). Moreover, studies with a 6-amino acid peptide that mimics the sequence around the phosphotyrosine residue 537 showed that it disrupted the Src–ER interaction and blocked breast cancer growth in vitro and in mice (35). Therefore, PTPH1 may additionally increase breast antihormone sensitivity by acting on additional tyrosine residues such as Y52 and/or Y219 and/or through regulating ER/Src interactions. Future clinical analyses are also warranted to establish whether there is a correlation between increased PTPH1 and decreased p-ER/Y537 protein expression in clinical breast cancer specimens and whether or not this inversely relationship predicts an improved outcome of antihormone therapy. These studies will provide additional evidence to indicate that PTPH1 may indeed increase breast cancer antihormone sensitivity through targeting ER/Y537 dephosphorylation.

Our studies may be the first that has established a true signaling connection between ER dephosphorylation and phosphatase activity in regulating breast cancer antihormone sensitivity. Although the serine–threonine phosphatase PP2A increases ER mRNA stability, whether it can induce ER dephosphorylation has not been demonstrated (36). In addition, although the dual specificity phosphatase 22 (DUSP22 or LMW-DSP2) decreases levels of p-ER/S118 expression in cells, whether this dephosphorylation can occur in vitro and whether this reaction impacts breast cancer growth and/or antihormone sensitivity remain all unknown (37). Our studies, on the other hand, showed that PTPH1 dephosphorylates ER/Y537 in vitro and in cells with and without estrogen stimulation, indicating its active role in maintaining low levels of intrinsic as well as ligand-induced p-ER/Y537 expression. Moreover, PTPH1 increases breast cancer growth in vitro (22) and in vivo (Fig. 3), which may also contribute to increased sensitivity of breast cancer to antiestrogens in addition to its regulatory activity on ER/Y537 dephosphorylation and ER nuclear accumulation. These results, together with its phosphatase activity-dependent stimulation of TAM-induced growth inhibition and of ER nuclear accumulation, highlight an application potential of PTPH1 in increasing breast cancer sensitivity to antiestrogens by dephosphorylating ER/Y537 through increased ER nuclear concentration.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: P.S. Suresh, G. Chen
Development of methodology: G. Chen
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