# Activation of Src by Protein Tyrosine Phosphatase 1B Is Required for ErbB2 Transformation of Human Breast Epithelial Cells

**Luis E. Arias-Romero,[1] Sayanti Saha,[1] Olga Villamar-Cruz,[1] Shu-Chin Yip,[1] Stephen P. Ethier,[2] Zhong-Yin Zhang,[3] and Jonathan Chernoff[1]**

[1]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]Karmanos Cancer Institute, Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan; and [3]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana

## Abstract

**Protein tyrosine phosphatase (PTP) 1B plays a major role in inhibiting signaling from the insulin and leptin receptors. Recently, PTP1B was found to have an unexpected positive role in ErbB2 signaling in a mouse model of breast cancer, but the mechanism underlying this effect has been unclear. Using human breast epithelial cells grown in a three-dimensional matrix, we found that PTP1B, but not the closely related enzyme T-cell PTP, is required for ErbB2 transformation *in vitro*. Activation of ErbB2, but not ErbB1, increases PTP1B expression, and increased expression of PTP1B activates Src and induces a Src-dependent transformed phenotype. These findings identify a molecular mechanism by which PTP1B links an important oncogenic receptor tyrosine kinase to signaling pathways that promote aberrant cell division and survival in human breast epithelial cells.** [Cancer Res 2009;69(11):4582–8]

## Introduction

Protein tyrosine phosphatase (PTP) 1B is an abundant cytoplasmic enzyme that plays a major role in down-regulating insulin and leptin signaling (1). PTP1B binds to and dephosphorylates the insulin receptor, thus terminating signals from this receptor tyrosine kinase. Deletion of the *Ptp1b* gene in mice causes hypersensitivity to insulin and is associated with marked increases in tyrosine phosphorylation of the insulin receptor and its targets (2, 3). PTP1B may also inhibit signaling from other receptor tyrosine kinases such as the platelet-derived growth factor and hepatocyte growth factor receptors (4–7). Consistent with its role as an inhibitor of receptor tyrosine kinases, overexpression of PTP1B in fibroblasts inhibits transformation by oncogenes that increase tyrosine phosphorylation, including ErbB2, Src, Bcr-Abl, and Crk, and also by Ras (8–11). In Src or Crk transformed cells, overexpression of PTP1B is associated with loss of tyrosine phosphorylation of key signaling proteins such as Crk and $p130^{Cas}$(10).

The above results, coupled with effects of PTP1B on the insulin receptor, gave rise to the idea that the main role of PTP1B is to act as a brake to proliferative and metabolic signals. However, recent data indicate that this idea is too limited. In fibroblasts, PTP1B is required for the activation of the small GTPases Ras (12) and Rac (13), enzymes that are generally associated with increased cell proliferation and motility. In addition, PTP1B has also been shown to activate Src by dephosphorylating the inhibitory Y527 site in the COOH terminus of this kinase (13–16). Thus, at endogenous levels of expression, PTP1B has certain progrowth properties in marked contrast to its aforementioned ability to revert transformation by various oncogenes when overexpressed. *In vivo, $Ptp1b^{-/-}$* mice resist transformation by the ErbB2 (HER-2/*neu*) oncogene, and transgenic overexpression of PTP1B in mammary cells is oncogenic (17, 18). Moreover, in a significant fraction of human clinical samples of ErbB2-positive breast cancer, amplification of chromosome 20q13 (containing the PTP1B gene) has been noted, with increased expression of PTP1B (19, 20). These findings imply that this enzyme plays a positive role in growth signaling in at least some tissues and that PTP1B might therefore serve as a therapeutic target in certain malignancies.

How does PTP1B contribute to oncogenesis in mammary cells? PTP1B is known to up-regulate two growth-promoting pathways: it activates Src and deactivates $p62^{Dok}$, an inhibitor of the Ras/mitogen-activated protein kinase pathway (21). Regarding these substrates, it is at least theoretically plausible that the reported *in vivo* effects of PTP1B loss on ErbB2-driven breast cancer can be explained in terms of failure to activate Src. However, Kaminski and colleagues have recently reported that Src activity is dispensable for ErbB2-driven carcinogenesis (22). Another PTP1B substrate, $p62^{Dok}$, represents an alternate explanation for the positive effects of PTP1B on transformation. When tyrosine phosphorylated, this protein complexes with, and activates, $p120^{RasGAP}$, leading to decreased Ras and mitogen-activated protein kinase activity (23, 24). Loss of PTP1B leads to hyperphosphorylation of $p62^{Dok}$, with consequent inactivation of Ras and its downstream effectors. However, tissue samples from ErbB2/*$Ptp1b^{-/-}$* mice gave inconsistent results regarding the tyrosine phosphorylation status of $p62^{Dok}$ (17, 18). For these reasons, whether the observed effects of PTP1B on ErbB2-driven carcinogenesis are due to the interactions of PTP1B with Src, $p62^{Dok}$, or unidentified substrates is not known.

In this article, we sought to clarify the molecular mechanism(s) by which PTP1B contributes to ErbB2 signaling in breast epithelial cells. We used a three-dimensional *in vitro* model to recapitulate the architectural elements of breast acinar development while retaining the ability to manipulate and analyze the pathways that underlie the effects of PTP1B on ErbB2 signaling. Consistent with its proposed role in oncogenic signaling, we found that activation of ErbB2, but not ErbB1, increased PTP1B expression and that small interfering RNA (siRNA)-induced reduction in PTP1B expression or inhibition of PTP1B activity by small-molecule inhibitors impeded the ability of activated ErbB2 to transform these cells and to activate Src and its associated downstream

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

S. Saha, O. Villamar-Cruz, and S-C. Yip contributed equally to this work.

**Requests for reprints:** Jonathan Chernoff, Fox Chase Cancer Center, Philadelphia, PA. Phone: 215-728-5319; Fax: 215-28-3616; E-mail: J_Chernoff@fccc.edu.

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doi:10.1158/0008-5472.CAN-08-4001

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signaling targets. In addition, we found that the suppressive effects of PTP1B loss could be bypassed by expression of mutationally activated Src. Likewise, we found that overexpression of PTP1B in breast epithelial cells distorted normal acinar morphology, causing unchecked proliferation, and loss of polarity. These effects were associated with Src activation and required the presence of protein phosphatase activity and the Src-binding motif in the COOH terminus of PTP1B. These results support a model in which PTP1B, by activating Src, cooperates with ErbB2 in transforming mammary epithelial cells.

## Materials and Methods

**Materials.** Anti-ERK, anti-Akt, anti-Src, phospho-ERK, phospho-Akt, phospho-Src, phospho-Y1248 ErbB2, phospho-Y1068 ErbB1, and phospho-Y416 and Y527 Src antibodies were purchased from Cell Signaling Technology. Monoclonal anti-hemagglutinin (12CA5) and anti-phosphotyrosine (PY20) antibodies were from BabCo and Transduction Laboratories, respectively. Anti-Ki-67 was from Santa Cruz Biotechnology. Monoclonal anti-T-cell PTP (TC-PTP) 3E2 (25) was a kind gift from Michel Tremblay. Reconstituted basement membrane (Matrigel) was from BD Life Science. PP2 was purchased from Calbiochem.

**Cell lines and three-dimensional cell culture.** 10A.ErbB2 cells (MCF-10A cells expressing a chimeric form of ErbB2; ref. 26), 7.ErbB2 cells (created by stable transduction of MCF7 cells with pMN.B2.F2.HA), and 10A.ErbB1 cells (created by stable transduction of MCF-10A cells with pMN.B1.F2.HA; ref. 26) were maintained in DMEM/F-12 (Life Technologies) supplemented with 5% donor horse serum, 20 ng/mL EGF (Harlan Bioproducts), 10 mg/mL insulin (Sigma), 1 ng/mL cholera toxin (Sigma), 100 mg/mL hydrocortisone (Sigma), 50 units/mL penicillin, and 50 mg/mL streptomycin. For three-dimensional cultures, ~5,000 cells were plated atop reconstituted basement membrane in 8-well slide chambers as described (26). To activate chimeric ErbB proteins, 1 mmol/L AP1510 was added to the growth medium. BT-474, MDA-MB-231, and SUM190 cells were grown in RPMI, 10% fetal bovine serum and Ham's F12, 10% fetal bovine serum, respectively.

**Expression plasmids, transfection, and transduction.** The retroviral expression vector pMN.B1.F2.HA, encoding ErbB1, was obtained from Muthuswamy and colleagues (26) and pWZL-PTP1B (5) was obtained from Ben Neel. The $PTP1B^{P309,310A}$(PTP-PA) and $PTP1B^{D161A}$ (PTP-DA) mutants were subcloned into pWZL from pCMV6H (10). To generate viral stocks, these vectors were transiently transfected into the Phoenix-Eco retroviral packaging line,[4] and supernatants were collected 48 h later. For retroviral transduction, 10A.ErbB2 cells were plated at $3 \times 10^5$ per 10 cm diameter dish and then infected with high-titer retroviruses and screened by anti-hemagglutinin or anti-PTP1B immunoblots.

**RNA interference.** The siRNA duplex targeted to PTP1B had the sequence (sense) 5′-CUUCCUAAGAACAAAAACCdTdT-3′ and (antisense) 5′-GGUUUUUGUUCUUAGGAAGdTdT-3′. The siRNA duplexes targeted to TC-PTP comprised a mixture of four different oligonucleotides (*SMART* pool). Equal amounts of sense and antisense RNA oligonucleotides were mixed and annealed according to the manufacturer's instructions. 10A.ErbB2 cells were transfected with 40 μL of 20 μmol/L siRNA for PTP1B or TC-PTP or the control siRNA duplex with Lipofectamine 2000 reagent for 12 h. All siRNAs were purchased from Dharmacon Research.

**Immunoprecipitation, immunoblotting, and in-cell Westerns.** Cells were lysed in a buffer containing 50 mmol/L Tris-HCl (pH 8.0), 137 mmol/L NaCl, 10% glycerol, 1% NP-40, 50 mmol/L NaF, 10 mmol/L β-glycerophosphate, 2 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride, and 10 μg/mL aprotinin. Lysates were clarified by centrifugation at 13,000 rpm for 10 min and protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce Chemical). For immunoprecipitations, lysates were incubated with the appropriate antibodies for 3 h overnight at 4°C. Immune complexes were collected onto protein A-Sepharose beads, washed extensively, resolved by SDS-PAGE, and transferred onto Immobilon-P membranes (Millipore). Immunoblots were blocked with 5% bovine serum albumin or Carnation nonfat dry milk in 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, and 0.05% Tween-20. After incubation with appropriate primary and secondary antibodies, blots were visualized using enhanced chemiluminescence reagents (Amersham Biosciences). Quantification was carried out using NIH ImageJ software version 1.40; data are expressed as relative units of phosphorylated protein per total protein for each band. Anti-PTP1B and anti-hemagglutinin antisera were used at 1:1,000 for immunoblotting. Anti-human PTP1B monoclonal antibodies (FG6) were used at 1 μg/mL for immunoprecipitations and immunoblotting. All other antibodies were used at concentrations as recommended by the supplier.

In-cell Westerns were done according to the manufacturer's specifications.[5] In brief, cells were fixed in 4% methanol-free formaldehyde in PBS, rinsed with PBS, blocked with 5% nonimmune rabbit or mouse serum in PBS/0.1% Triton X-100 for 1 h, and incubated with primary antibodies for 2 h at 37°C. The cells were then rinsed three times in PBS for 5 min each and then incubated in fluorochrome-conjugated secondary antibody diluted 1:500 (Alexa Fluor 680) or 1:800 (IRDye 800CW) in PBS/0.1% Triton X-100 for 1 h at room temperature in the dark. The cells were then rinsed three times in PBS for 5 min each and scanned using a Li-Cor Odyssey device.

**Immunofluorescence analysis.** The acinar structures were fixed in 2% paraformaldehyde at room temperature for 15 min and processed as described by Muthuswamy and colleagues (26). Confocal analyses were done with a Nikon TE2000 confocal microscopy system.

## Results

**PTP1B is required for ErbB2-mediated transformation of MCF-10A cells.** To establish the functions of PTP1B in human breast epithelial cells, we examined the effects of this phosphatase in ErbB2 signaling in MCF-10A cells grown in three-dimensional conditions. MCF-10A cells are immortalized, nontransformed cells derived from a reduction mammoplasty, which form organized acini when grown within three-dimensional matrices such as reconstituted basement membrane (26, 27). In MCF-10A cells that stably express an AP1510-activatable, chimeric form of ErbB2 (10A.ErbB2 cells), treatment with AP1510 caused characteristic changes in acinar morphogenesis, proliferation, and luminal apoptosis, resembling those seen in human ductal carcinoma of the breast (ref. 26; Fig. 1*A*).

Next, we asked if endogenous PTP1B is required for the phenotypic effects of ErbB2 on acinar development. We used siRNA to reduce PTP1B expression in 10A.ErbB2 cells and assessed the effects on acinar morphogenesis. Transfection of 10A.ErbB2 cells with siRNA directed against PTP1B resulted in ~80% loss of PTP1B expression, whereas a scrambled siRNA did not affect PTP1B expression (Fig. 1*C*). Reduction of PTP1B did not have a notable effect on basal rates of cell proliferation or apoptosis (data not shown), nor did it affect acinar architecture; however, the phenotypic effects of ErbB2 activation were blocked in cells treated with siRNA against PTP1B (Fig. 1*B*). The siRNA against PTP1B did not affect ErbB2 kinase activity (Fig. 1*D*), indicating that the suppression of the multiacinar phenotype was mediated by events downstream of this receptor tyrosine kinase.

TC-PTP is a close relative of PTP1B, and we therefore asked if this phosphatase also affects ErbB2 signaling in 10A.ErbB2 cells.

[4] http://www.stanford.edu/group/nolan/index.html

[5] http://www.cellsignal.com/support/protocols/In_Cell_Western.html

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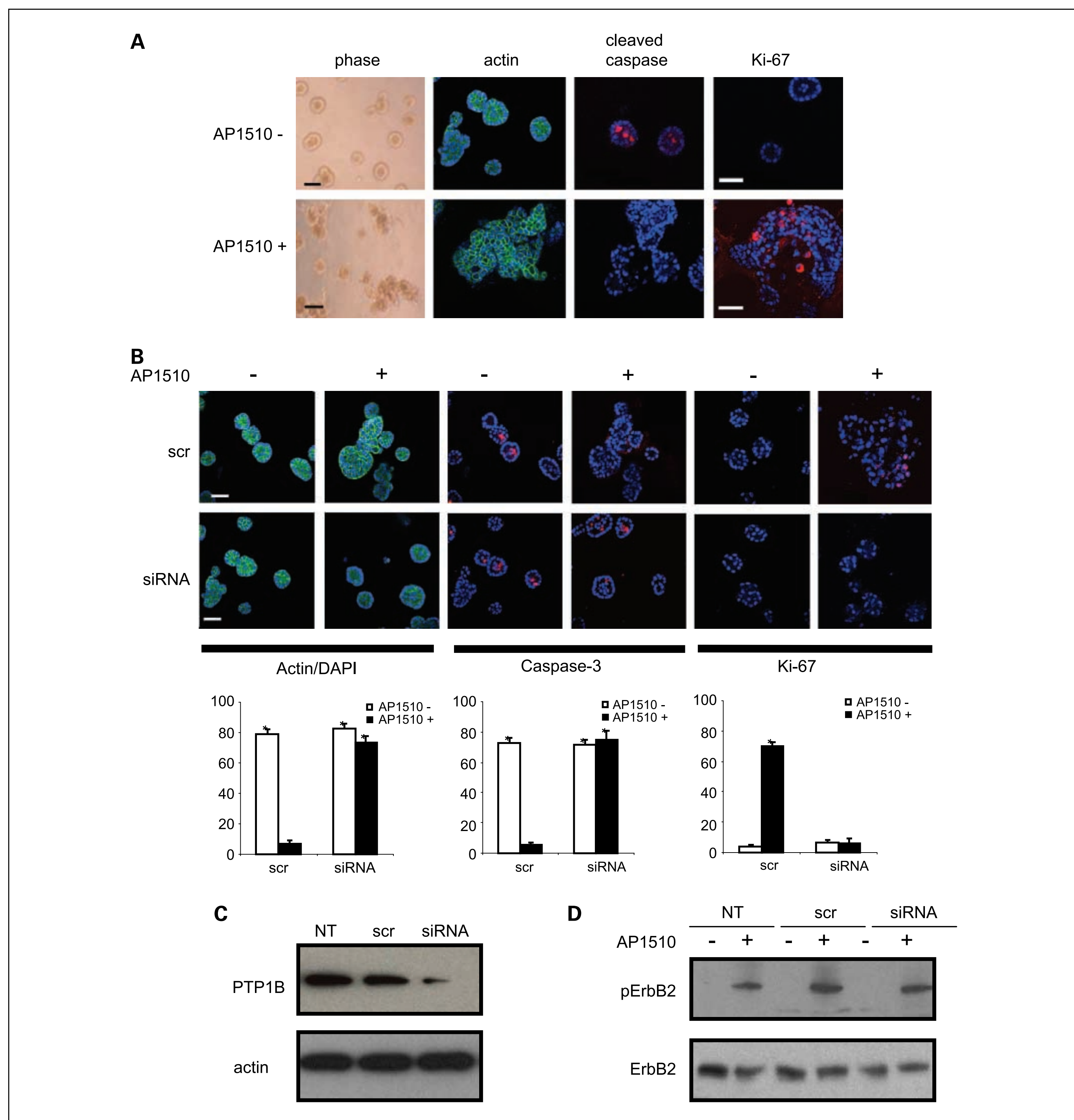


**Figure 1.** PTP1B is required for ErbB2-mediated transformation of MCF-10A cells. *A,* MCF10A.ErbB2 cells were plated atop reconstituted basement membrane. Beginning on day 3, 1 mmol/L AP1510 was added to the medium. The cells were fixed on day 12 and stained with Oregon green-phalloidin, Ki-67, or anti-cleaved caspase-3. *B,* MCF10A.ErbB2 cells were transfected with control or PTP1B-specific siRNA and plated atop reconstituted basement membrane and processed as in *A*. Percentage of unilamellar acini, Ki-67-positive, and anti-cleaved caspase-3-positive acini were scored based on assessment of 50 to 60 acini per well. *C,* anti-PTP1B immunoblot. *D,* blot of activated ErbB2 and total ErbB2.

We used siRNA to knock down TC-PTP expression and assessed its effect on acinar development in three-dimensional cultures. Despite effective reduction in TC-PTP expression by siRNA, acini developed normally and were disrupted by activation of ErbB2 (Supplementary Fig. S1A). Thus, the effects of PTP1B on ErbB2 signaling are not shared with its closest relative, TC-PTP.

To rule out off-target effects and also to ensure that these effects were due to loss of PTP1B activity rather than loss of the protein, we carried out similar experiments using two different, highly specific small-molecule inhibitors of PTP1B, compounds II and III (28). Compounds II and III are cell-permeable analogues of the most potent and selective PTP1B inhibitor (compound 40 in ref. 29)

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reported to date, which displays a $K_i$ value of 2.4 nmol/L for PTP1B and exhibits several orders of magnitude of selectivity in favor of PTP1B against a panel of PTPs (29). As with siRNA, chemical inhibition of PTP1B suppressed the multiacinar effects of ErbB2 (Fig. 2). Together with the siRNA data, these experiments show that PTP1B function is required by ErbB2 to induce a multiacinar phenotype.

**Molecular pathways affected by PTP1B in MCF-10A cells.** We next tested if PTP1B links ErbB2 to Src in MCF-10A cells. First, we assessed Src activity in response to ErbB2. Three-dimensional cultures of 10A.ErbB2 cells were treated with AP1510 and Src activity assessed by immunoblot using phosphospecific antibodies directed against $Tyr^{416}$ in Src. As expected, activation of ErbB2 induced activation of Src (Fig. 3). The expected reciprocal changes were seen in Src $Tyr^{527}$ phosphorylation: AP1510-induced decreased Src Y527 phosphorylation (Supplementary Fig. S2A). In contrast, in cells treated with siRNA against PTP1B, or a small-molecule PTP1B inhibitor, Src activation by ErbB2 was markedly suppressed as assessed by decreased $Tyr^{416}$ and increased $Tyr^{527}$ phosphorylation (Fig. 3*A*; Supplementary Fig. S2A). Similar results were seen regarding two other important signaling molecules that are activated by Src, Akt and ERK (Fig. 3*B* and *C*). Notably, inhibition of PTP1B did not indiscriminately affect other ErbB2-induced signaling events, such as the tyrosine phosphorylation of Shc, nor did it affect total tyrosine phosphorylation (Supplementary Fig. S2B and C). Also, the effects of PTP1B on ErbB2 signaling were not confined to 10A.ErbB2 cells. As in 10A.ErbB2 cells, addition of the specific PTP1B inhibitor compound II to the ErbB2-expressing human breast cancer cell lines BT-474, MDA-MB-231, or SUM190 resulted in marked loss of ERK activity (Supplementary Fig. S3).

**Activated Src bypasses requirement for PTP1B in ErbB2-mediated transformation.** Treatment of 10A.ErbB2 cells with the Src inhibitor PP2 blocked the effects of ErbB2 activation on acinar morphology (Fig. 4*A*). If Src activation downstream of ErbB2 and PTP1B mediates the effects of ErbB2 on signal transduction, then a constitutively active form of Src should bypass the need for either ErbB2 or PTP1B in signaling, and loss of Src should block the actions of these enzymes. We transduced 10A.ErbB2 cells with $Src^{Y527F}$, which lacks the inhibitory site of tyrosine phosphorylation in the COOH terminus and is therefore constitutively active. These cells display aberrant acini and elevated Akt and ERK activity even in the absence of activated ErbB2 (Fig. 4*B*). In addition, these

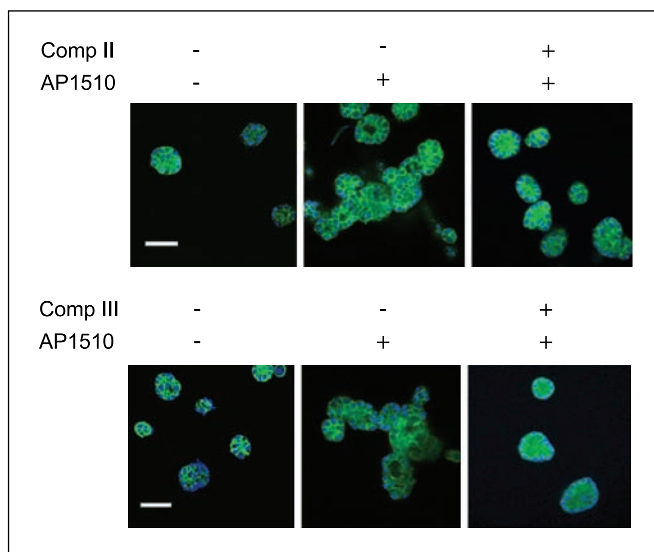


**Figure 2.** Small-molecule inhibition of PTP1B blocks ErbB2-mediated transformation of MCF-10A cells. MCF10A.ErbB2 cells plated atop reconstituted basement membrane were treated with vehicle, 25 nmol/L compound II (*Comp II*), or 250 nmol/L compound III (*Comp III*), plus 1 mmol/L AP1510, on day 3 as indicated, and fixed on day 12. Medium was replaced (with PTP1B inhibitors and AP1510) every 3 d.

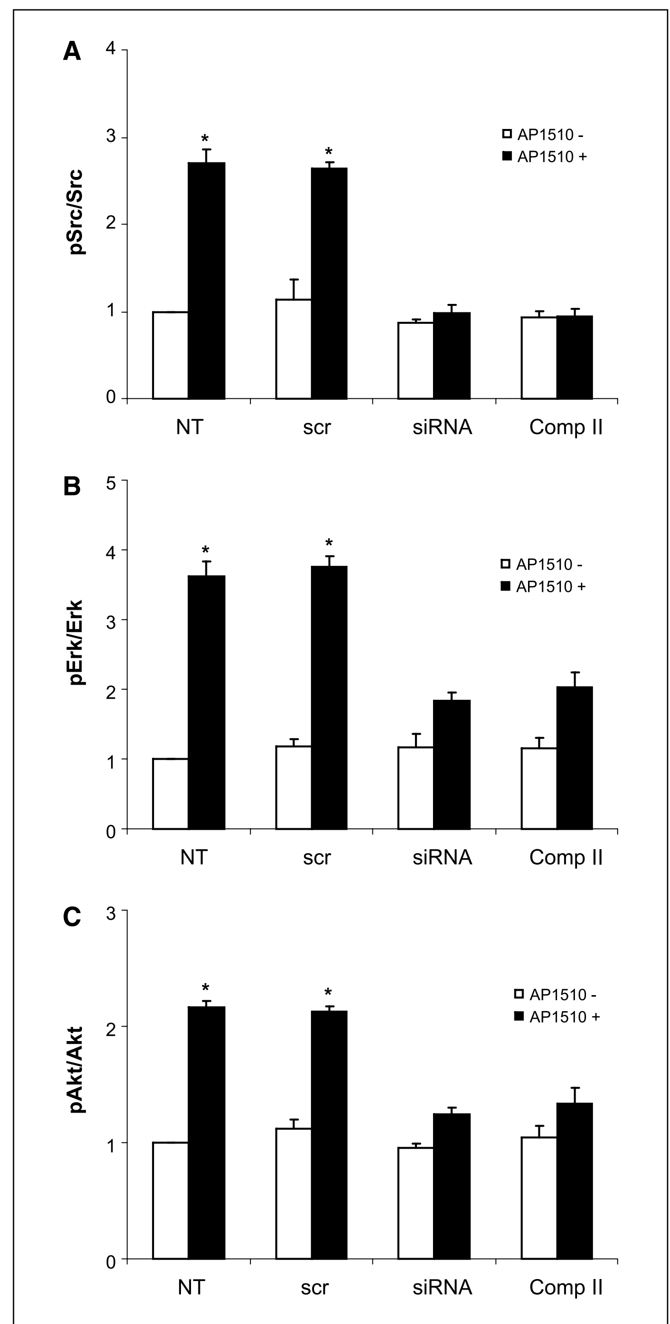


**Figure 3.** Molecular pathways affected by PTP1B in MCF-10A cells. MCF10A.ErbB2 cells were transfected with control or PTP1B-specific siRNA and plated atop reconstituted basement membrane. Cells were stimulated with vehicle or AP1510 on day 3 and fixed on day 12. Where indicated, cells were treated with specific small-molecule inhibitors of PTP1B or Src. The activity of Src, Akt, and ERK was assessed by in-cell Western using phosphospecific antibodies.

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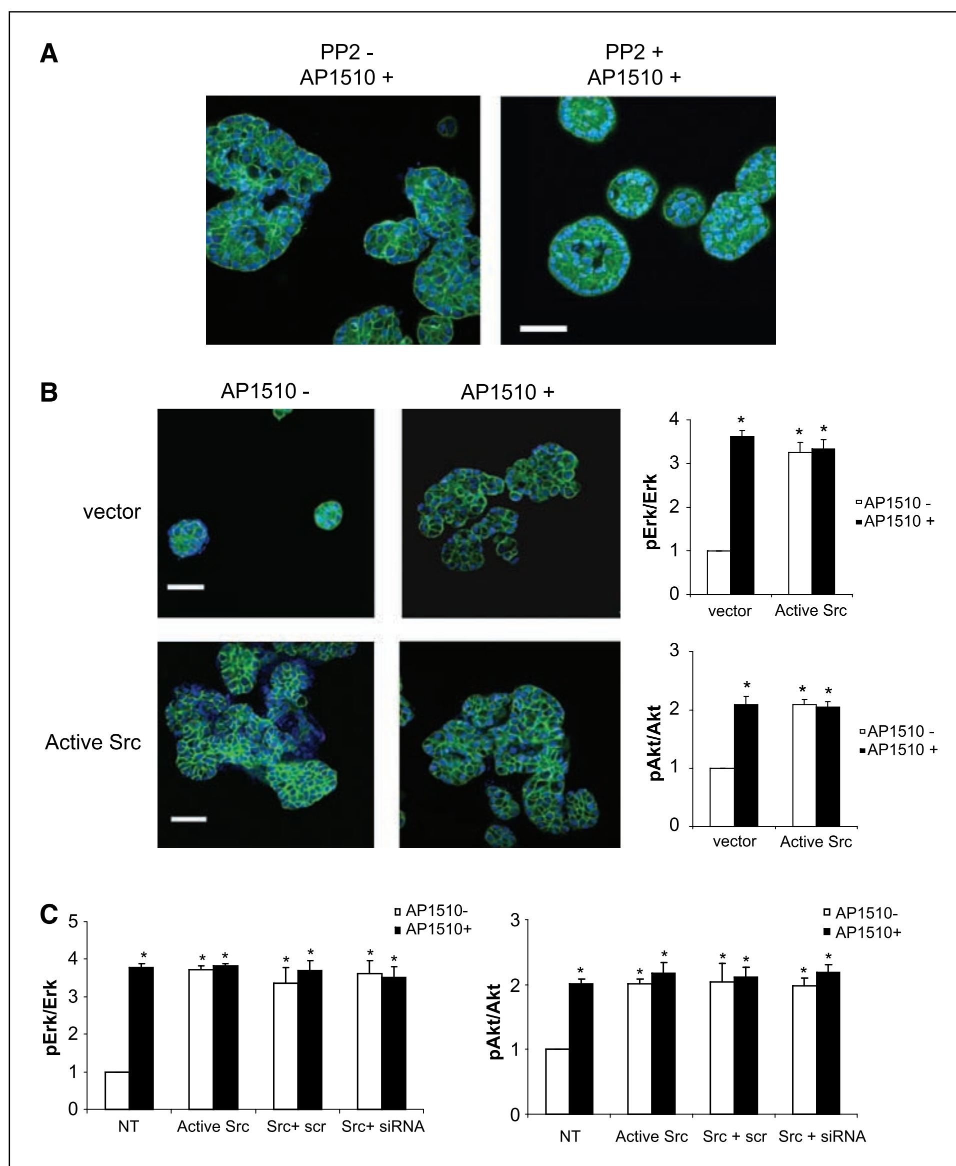


**Figure 4.** Activated Src bypasses requirement for PTP1B in Neu-mediated transformation. *A,* MCF10A.ErbB2 cells were treated with 10 mmol/L PP2 and 1 mmol/L AP1510. In-cell Western blots were used to determine the activity of Src, Akt, and ERK. *B,* MCF10A.ErbB2 cells were transduced with a control retrovirus or a retrovirus encoding activated c-Src. Acinar morphology and activity of Src, Akt, and ERK are shown. *C,* cells transduced with activated c-Src were transfected with scrambled siRNA or PTP1B-specific siRNA. The activities of Src, Akt, and ERK, as assessed by in-cell Westerns, are shown.

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effects were also seen in Src-expressing cells treated with an inhibitor of PTP1B (Fig. 4*C*).

**Overexpression of PTP1B distorts acinar development via activation of Src.** Interestingly, we found that the expression level of PTP1B increased markedly after treatment with AP1510 (Fig. 5*A*). Similar results were seen in MCF-7 cells expressing the same AP1510-activatable form of ErbB2 (data not shown), indicating that the effects on PTP1B are not confined to a single type of breast epithelial cell line. These results are in line with previous observations that ErbB2-transformed cells have elevated PTP1B expression and suggest a causal relationship between these events (30). In contrast, PTP1B expression was not altered in 10A.ErbB1 cells following activation of ErbB1, indicating that the effect on PTP1B expression was specific to ErbB2 and not a general response to growth stimuli (Fig. 5*A*). The ErbB2 effect on PTP1B expression was reversible; withdrawal of AP1510 resulted in the rapid decline in PTP1B expression (Supplementary Fig. S4).

If Src activation by PTP1B plays a role in ErbB2 signaling, then activation or overexpression of PTP1B should affect acinar development in a Src-dependent manner. To test this idea, we examined the effects of PTP1B overexpression in 10A.ErbB2 cells. These cells were infected with a retrovirus encoding no insert, wild-type PTP1B, PTP1B-PA (a mutant that cannot bind Src homology 3-domain containing proteins, including Src; ref. 10), or PTP1B-DA (a catalytically dead mutant). In all cases, ~3-fold elevation in PTP1B expression was observed, comparable with the degree of PTP1B expression in cells following AP1510 treatment (Fig. 5*B*). Overexpression of wild-type PTP1B, but not the PTP1B mutants, was associated with a multilaminar phenotype (Fig. 5*C*). The effects of wild-type PTP1B were blocked by the Src inhibitor PP2 (Fig. 5*D*), consistent with the idea that Src represents a key target of PTP1B in breast epithelial cells.

## Discussion

In these studies, we used a three-dimensional breast epithelial cell culture system to show that PTP1B plays a positive role in ErbB2 signaling. Using this model, we were able to dissect the

signaling events that underlie these important effects. We showed that one of the primary targets for PTP1B activity in human breast epithelial cells is the tyrosine kinase Src. This assertion rests on the following observations: (*a*) the ErbB2-induced multiacinar phenotype, characterized by unrestrained proliferation, luminal cell survival, and loss of polarity, is suppressed by decreased expression or chemical inhibition of PTP1B but not by decreased expression of the related phosphatase TC-PTP; (*b*) loss of PTP1B function blocks the activation of Src and downstream signaling pathways by ErbB2; (*c*) mutationally activated Src bypasses loss of PTP1B in ErbB2-mediated transformation; and (*d*) activation of ErbB2 leads to elevated expression of PTP1B and PTP1B overexpression distorts mediated acinar morphogenesis; these effects require an intact Src homology 3-binding motif and are blocked by a Src inhibitor. Taken together, these findings support a model in which PTP1B expression is increased by ErbB2 and that its activity is required to activate Src by ErbB2 and that such activation is required for transformation of breast epithelial cells.

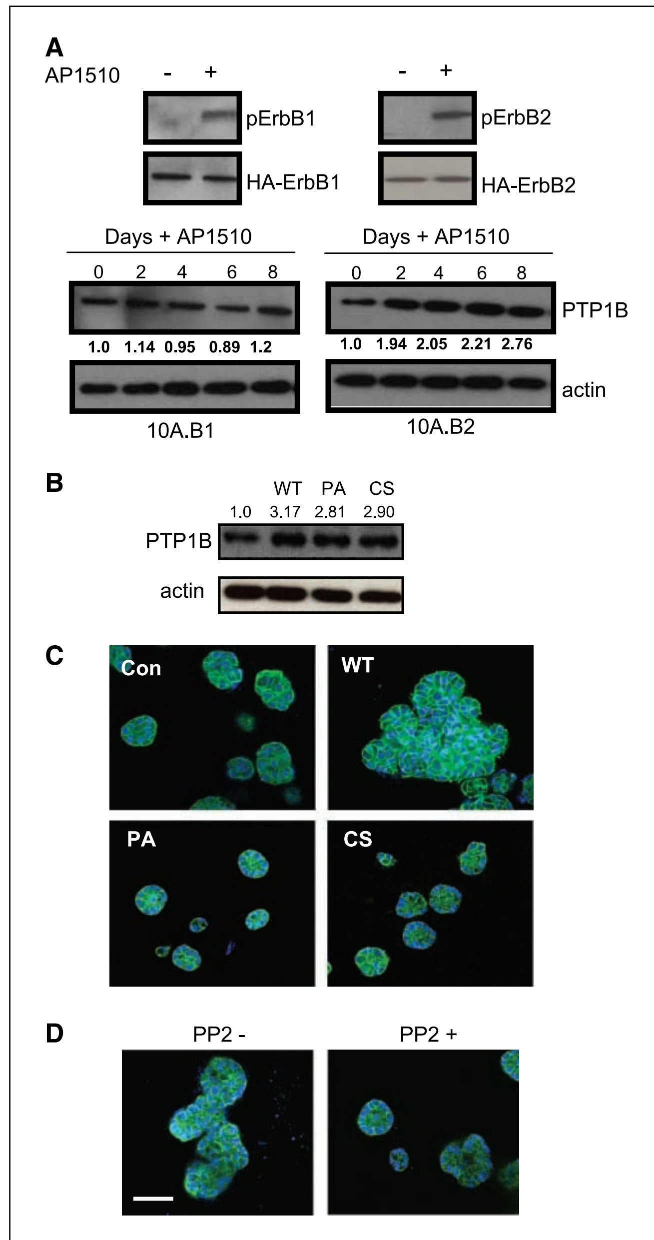


**Figure 5.** Overexpression of PTP1B alters acinar morphogenesis via activation of Src. *A,* anti-phospho-ErbB1 or ErbB2, anti-PTP1B, and anti-actin immunoblots from MCF10A.ErbB2 and MCF10A.ErbB1 cells, respectively, grown in the absence or presence of AP1510. Numbers indicate fold expression relative to unstimulated ErbB1 or ErbB2. *B,* a retrovirus bearing wild-type or mutant forms of PTP1B was used to infect MCF10A.ErbB2 cells, which were then plated atop reconstituted basement membrane. Cells were fixed on day 12. An anti-PTP1B immunoblot is shown. Numbers indicate fold expression relative to endogenous PTP1B. *C,* 4′,6-diamidino-2-phenylindole and Oregon green-phalloidin staining of acini overexpressing the indicated forms of PTP1B. *D,* effects of the Src inhibitor PP2 on wild-type PTP1B-induced changes in acinar morphology.

These findings are compatible with recent *in vivo* experiments in which loss of PTP1B was shown to reduce, and transgenic overexpression increase, mammary oncogenesis in mice (17, 18). However, our results contrast with earlier work from our laboratory and others that showed that PTP1B overexpression suppresses transformation by several oncogenes, including ErbB2 and Src (8, 10, 11). How can these results be reconciled? One explanation may lie in the choice of cells for analysis. In all of the prior work, transformation was assayed in mouse or rat fibroblasts as opposed to human mammary cells. It is well established that transformation of human and murine cells has distinct signaling requirements (31, 32). In addition, epithelial cells likewise use different signaling networks than fibroblasts. For these reasons, it is likely that the observed effects of PTP1B on ErbB2 signaling in human MCF-10A cells are germane to breast epithelial cells and thus to breast cancer but do not apply to all cell types.

We showed that PTP1B is required for Src activation by ErbB2 in MCF-10A cells and that activated Src bypasses the need for PTP1B in ErbB2-mediated transformation. These results suggest that Src is a key target in mediating the positive role of PTP1B in ErbB2 oncogenesis. The role of Src in ErbB2 signaling has been controversial. On the one hand, Src activation strongly correlates with ErbB2 overexpression in human ductal carcinoma *in situ* (33), and Src associates with activated ErbB2 in human breast cancer cells in a Src homology 2 domain-dependent manner, resulting in activation of Src (34). In addition, inhibition of Src in breast epithelial cells suppresses ErbB2-mediated motility and soft-agar growth (34–36). All of these studies suggest that Src plays an important role in ErbB2 oncogenic signaling. On the other hand, transgenic overexpression of the Csk gene, which encodes a protein kinase that suppresses Src activation, does not affect ErbB2-mediated breast cancer in mice (22). These latter findings point to the possibility of a nonenzymatic role for Src in ErbB2 signaling. Our findings are not inconsistent with this idea, because activation of Src by PTP1B-catalyzed dephosphorylation of Y527, in addition to its effects on kinase activity, also leads to conformational changes in Src that could expose scaffolding motifs.

In clinical samples, it has been long noted that PTP1B expression increases in several human tumor types, including breast and ovarian cancers (20, 37). Furthermore, PTP1B expression is elevated in breast epithelial cells transformed by ErbB2 (refs. 18, 30; Fig. 5*A*). It has been proposed that this increase in expression represents the attempt of cells to counterincreased PTK activity. In light of data that PTP1B is required for Ras and Rac activation *in vitro*, and recent data that PTP1B is required for transformation by Neu in mouse models of breast cancer, it is more likely that elevated PTP1B contributes to transformation in certain human cancers.

Given that loss of PTP1B function attenuates carcinogenesis in mouse models of breast cancer, it is plausible that PTP1B inhibitors might prove useful in the therapy of certain cancers.

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Such a notion would have seemed heretical just a few years ago, as copious previous data using overexpressed PTP1B in fibroblasts in culture indicated that this protein acts as a suppressor of growth factor signaling and that its loss might therefore be expected to augment cell proliferation. Instead, the opposite seems to be true in the case of the oncogene ErbB2 both in our three-dimensional cell culture system and in ErbB2-driven transgenic mouse models of breast cancer. Evidently, in these systems, the effects of PTP1B on receptor tyrosine kinases are either confined to the insulin receptor or are more than offset by positive effects on other signaling elements such as Src. These findings highlight the necessity of combining advanced cell-based and animal models when probing complex signal transduction pathways.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

Received 10/16/08; revised 3/23/09; accepted 3/24/09; published OnlineFirst 5/12/09.

**Grant support:** NIH grants R01 CA58836 (J. Chernoff), R01 CA69202 (Z-Y. Zhang), R01 CA100724 (S.P. Ethier), and F32 DK079474 (S-C. Yip), Fox Chase Cancer Center grant P30 CA006927, and an appropriation from the State of Pennsylvania.

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We thank Senthil Muthuswamy, Ben Neel, Michel Tremblay, and Ariad Pharmaceuticals for the gifts of 10A.ErbB2 cells, plasmid pWZL-PTP1B, monoclonal antibody 3E2, and AP1510, respectively, and Erica Golemis for comments on the article.

## References

1. Bourdeau A, Dube N, Tremblay ML. Cytoplasmic protein tyrosine phosphatases, regulation and function: the roles of PTP1B and TC-PTP. Curr Opin Cell Biol 2005;17:203–9.
2. Elchebly M, Payette P, Michaliszyn E, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999; 283:1544–8.
3. Klaman LD, Boss O, Peroni, OD, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 2000;20:5479–89.
4. Liu F, Chernoff J. PTP1B associates with and is tyrosine phosphorylated by epidermal growth factor receptor in cultured cells. Biochem J 1997;327:139–45.
5. Haj FG, Markova B, Klaman LD, Bohmer FD, Neel BG. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B. J Biol Chem 2003;278: 739–44.
6. Lammers R, Bossenmaier B, Cool DE, et al. Differential activities of protein tyrosine phosphatases in intact cells. J Biol Chem 1993;268:22456–62.
7. Kakazu A, Sharma G, Bazan HE. Association of protein tyrosine phosphatases (PTPs)-1B with c-Met receptor and modulation of corneal epithelial wound healing. Invest Ophthalmol Vis Sci 2008;49:2927–35.
8. Brown-Shimer S, Johnson KA, Hill DE, Bruskin AM. Effect of protein tyrosine phosphatase 1B expression on transformation by the human neu oncogene. Cancer Res 1992;52:478–82.
9. LaMontagne KRJ, Hannon G, Tonks NK. Protein tyrosine phosphatase PTP1B suppresses p210 bcr-abl-induced transformation of rat-1 fibroblasts and promotes differentiation of K562 cells. Proc Natl Acad Sci U S A 1998;95:14094–9.
10. Liu F, Chernoff J. Suppression of oncogene-mediated transformation of rat 3Y1 fibroblasts by protein tyrosine phosphatase 1B requires a functional SH3-ligand. Mol Cell Biol 1998;18:250–9.
11. Woodford-Thomas TA, Rhodes JD, Dixon JE. Expression of a protein tyrosine phosphatase in normal and v-src-transformed mouse 3T3 fibroblasts. J Cell Biol 1992; 117:401–14.
12. Dube N, Cheng A, Tremblay ML. The role of protein tyrosine phosphatase 1B in Ras signaling. Proc Natl Acad Sci U S A 2004;101:1834–9.
13. Dadke S, Chernoff J. Protein-tyrosine phosphatase 1B mediates the effects of insulin on the actin cytoskeleton in immortalized fibroblasts. J Biol Chem 2003;278: 40607–11.
14. Bjorge JD, Pang A, Fujita DJ. Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. J Biol Chem 2000;275:41439–46.
15. Anderie I, Schulz I, Schmid A. Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets. Cell Signal 2007;19:582–92.
16. Liang F, Lee SY, Liang J, Lawrence DS, Zhang ZY. The role of protein-tyrosine phosphatase 1B in integrin signaling. J Biol Chem 2005;280:24857–63.
17. Julien SG, Dube N, Read M, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis. Nat Genet 2007;39:338–46.
18. Bentires-Alj M, Neel BG. Protein-tyrosine phosphatase 1B is required for HER2/Neu-induced breast cancer. Cancer Res 2007;67:2420–4.
19. Tanner MM, Tirkkonen M, Kallioniemi A, et al. Independent amplification and frequent co-amplification of three nonsyntenic regions on the long arm of chromosome 20 in human breast cancer. Cancer Res 1996;56:3441–5.
20. Wiener JR, Kerns BJ, Harvey EL, et al. Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression. J Natl Cancer Inst 1994;86:372–8.
21. Tonks NK, Muthuswamy SK. A brake becomes an accelerator: PTP1B-a new therapeutic target for breast cancer. Cancer Cell 2007;11:214–6.
22. Kaminski R, Zagozdzon R, Fu Y, et al. Role of SRC kinases in Neu-induced tumorigenesis: challenging the paradigm using Csk homologous kinase transgenic mice. Cancer Res 2006;66:5757–62.
23. Yamanashi Y, Baltimore D. Identification of the Abl-and rasGAP-associated 62 kDa protein as a docking protein, Dok. Cell 1997;88:205–11.
24. Kashige N, Carpino N, Kobayashi R. Tyrosine phosphorylation of p62dok by p210bcr-abl inhibits RasGAP activity. Proc Natl Acad Sci U S A 2000;97: 2093–8.
25. Ibarra-Sanchez MJ, Wagner J, Ong MT, Lampron C, Tremblay ML. Murine embryonic fibroblasts lacking TC-PTP display delayed G1 phase through defective NF-B activation. Oncogene 2001;:4728–39.
26. Muthuswamy SK, Li D, Lelievre S, Bissell MJ, Brugge JS. ErbB2, but not ErbB1, reinitiates proliferation and induces repopulation in epithelial acini. Nat Cell Biol 2001;3:785–92.
27. Soule HD, Maloney TM, Wolman SR, et al. Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res 1990;50:6075–86.
28. Zhang ZY. Functional studies of protein tyrosine phosphatases with chemical approaches. Biochim Biophys Acta 2005;1754:100–7.
29. Shen K, Keng YF, Wu L, Guo XL, Lawrence DS, Zhang ZY. Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure. J Biol Chem 2001;276: 47311–9.
30. Zhai YF, Beittenmiller H, Wang B, et al. Increased expression of specific protein tyrosine phosphatases in human breast epithelial cells neoplastically transformed by the neu oncogene. Cancer Res 1993;53: 2272–8.
31. Lim KH, Baines AT, Fiordalisi JJ, et al. Activation of RalA is critical for Ras-induced tumorigenesis of human cells. Cancer Cell 2005;7:533–45.
32. Hamad NM, Elconin JH, Karnoub AE, et al. Distinct requirements for Ras oncogenesis in human versus mouse cells. Genes Dev 2002;16:2045–57.
33. Wilson GR, Cramer A, Welman A, et al. Activated c-SRC in ductal carcinoma *in situ* correlates with high tumour grade, high proliferation and HER2 positivity. Br J Cancer 2006;95:1410–4.
34. Muthuswamy SK, Siegel PM, Dankort DL, Webster MA, Muller WJ. Mammary tumors expressing the neu proto-oncogene possess elevated c-Src tyrosine kinase activity. Mol Cell Biol 1994;14:735–43.
35. Sheffield LG. c-Src activation by ErbB2 leads to attachment-independent growth of human breast epithelial cells. Biochem Biophys Res Commun 1998;250: 27–31.
36. Belsches-Jablonski AP, Biscardi JS, Peavy DR, Tice DA, Romney DA, Parsons SJ. Src family kinases and HER2 interactions in human breast cancer cell growth and survival. Oncogene 2001;20:1465–75.
37. Tanner MM, Grenman S, Koul A, et al. Frequent amplification of chromosomal region 20q12–13 in ovarian cancer. Clin Cancer Res 2000;6:1833–89.

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