

A Brake Becomes an Accelerator: PTP1B—A New Therapeutic Target for Breast Cancer

Nicholas K. Tonks^{1,*} and Senthil K. Muthuswamy^{1,*}

¹Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

*Correspondence: tonks@cshl.edu, muthuswa@cshl.edu

DOI 10.1016/j.ccr.2007.02.022

The protein tyrosine phosphatase PTP1B, previously recognized for its role in downregulating insulin and leptin signaling, has now been shown to function as a positive regulator of signaling events associated with breast tumorigenesis. Inhibitors of PTP1B that have been developed as drug candidates for treatment of diabetes and obesity may offer new avenues for the treatment of breast cancer.

ErbB2 (HER2, Neu) is a protein tyrosine kinase (PTK) of the EGF receptor family that is overexpressed in ~25% of breast cancer, where it associated with poor prognosis. Characterization of the signaling events initiated by ErbB2, and the mechanisms by which they are regulated, would provide a platform for generating novel therapeutic strategies that could complement existing anti-ErbB2 treatments, such as Herceptin (Trastuzumab) (Hynes and Lane, 2005). Two recent papers have revealed an important role for PTP1B, the prototypic member of the protein tyrosine phosphatase (PTP) superfamily, as a positive mediator of the ErbB2-induced signals that trigger tumorigenesis and metastasis (Betires-Alj and Neel, 2007; Julien et al., 2007).

PTP1B has been implicated in downregulation of signaling through several RPTKs. In particular, *ptp1B* knockout mice display a striking phenotype—

enhanced sensitivity to insulin and resistance to obesity induced by a high-fat diet (Tonks, 2003). In effect, if you feed *ptp1B* knockout mice the equivalent of burgers and fries, they don't get fat! This phenotype reflects the abrogation of PTP1B's function

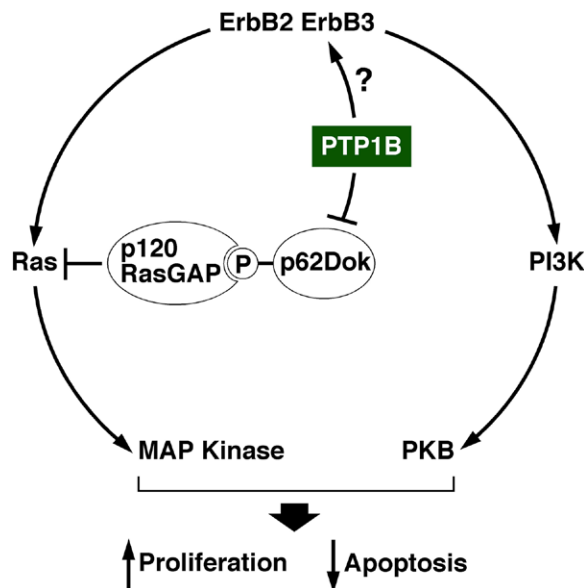


Figure 1. PTP1B Exerts a Positive Effect on ErbB2-Induced Tumorigenesis at the Level of the Ras/MAP Kinase and PI3 Kinase/PKB Signaling Pathways

The adaptor protein p62Dok acts as a negative regulator of the Ras/MAP kinase pathway and cell proliferation, at least in part through its ability to associate with the Ras GTPase-activating protein p120 RasGAP. It has been proposed that PTP1B dephosphorylates p62Dok, thereby attenuating its inhibitory effects on Ras activation and promoting MAP kinase signaling. PTP1B also plays a positive role in the phosphorylation and activation of PKB. Although ErbB3 is kinase defective, its intracellular segment becomes tyrosine phosphorylated upon heterodimerization with ErbB2, leading to recruitment and activation of PI3 kinase. Loss of PTP1B leads to reduced expression of ErbB3 and suppression of the phosphorylation and activation of PKB. However, the mechanism by which PTP1B exerts a positive effect on PI3 kinase signaling is unclear.

in downregulating signaling through insulin and leptin receptors. Consequently, the development of inhibitors of PTP1B has become a high priority in the pharmaceutical industry as a novel therapeutic strategy for the treatment of diabetes and obesity.

Nevertheless, the function of PTP1B is not restricted to metabolic regulation, and a complex mixture of positive and negative effects of the phosphatase on various tyrosine phosphorylation-dependent signaling pathways have been reported.

PTP1B is overexpressed in *erbB2*-transformed cell lines. In addition, the *ptp1B* gene is located at 20q13, a region that is frequently amplified in breast cancer and associated with poor prognosis, raising the question of the function of PTP1B in the etiology of breast cancer. In two separate studies, this question was addressed by crossing transgenic mice expressing activated forms of ErbB2 with *ptp1B* knockout animals. The first study utilized NDL2 mice, which express an activated mutant of ErbB2 bearing a five amino acid in-frame deletion in the extracellular segment under the control of the mouse mammary tumor virus (MMTV) promoter (Julien et al., 2007). These mice develop mammary

tumors that display features of human breast cancer. NDL2 were crossed with *ptp1B* knockout mice, both in FVB genetic background. Tumor development was delayed by ~85 days in the absence of PTP1B, with an intermediate delay (~35 days) in *ptp1B* hetero-

zygotes, consistent with dose-dependent effects of the phosphatase. In addition to the delay in tumor onset, both *ptp1B^{+/-}* and *ptp1B^{-/-}* mice displayed decreased incidence of lung metastases, suggesting a role for the phosphatase during development and progression of mammary tumors. The second study crossed MMTV-NeuNT mice expressing the transmembrane domain point mutant form of ErbB2 (V664Q) with *ptp1B* knockout mice, but on a mixed strain background (FVB × 129Sv:C57B6/J). In this case, the absence of PTP1B also led to a pronounced delay in tumor development, but no effect was observed in mice heterozygous for PTP1B (Betires-Alj and Neel, 2007). In contrast to the effects observed for MMTV-NeuNT, loss of PTP1B did not alter mammary tumorigenesis induced by polyoma middle T antigen, demonstrating specificity in the effects of the phosphatase.

The dephosphorylation and activation of Src family PTKs has been established as underlying the function of other positively acting PTPs, such as CD45, in different signaling contexts (Hermiston et al., 2003). Consistent with a positive signaling function, PTP1B has been identified as the major phosphatase catalyzing c-Src dephosphorylation in breast cancer cell lines (Bjorge et al., 2000). However, no change in phosphorylation or activation of c-Src was observed in these animal models of breast cancer, which is consistent with an earlier study indicating that the kinase function of Src is not required for ErbB2-induced tumorigenesis (Kaminski et al., 2006). Instead, both labs noted that loss of PTP1B is accompanied by attenuated activation of the Ras/MAP kinase pathway. The Tremblay group suggests that the mechanism involves dephosphorylation and inhibition of p62Dok, an adaptor protein previously identified as a substrate of PTP1B, which down-regulates Ras/MAP kinase signaling through its association with the Ras GTPase-activating protein p120RasGAP (Dube et al., 2004) (Figure 1). Enhanced phosphorylation of p62Dok in *ptp1B^{-/-}* tumors correlated

with decreased phosphorylation and increased levels of p120RasGAP protein (Julien et al., 2007). However, changes in p120RasGAP were not observed in the study by Neel's group (Betires-Alj and Neel, 2007). The reason for this discrepancy is unclear, but this and other differences between the conclusions of the two studies may reflect the differences in mouse strain background, homogeneous versus mixed, and possibly also the different forms of activated ErbB2 that were used.

ErbB2-induced mammary tumors express high levels of ErbB3, and heterodimerization of ErbB2 and ErbB3 is critical for signaling to the PI3 kinase/PKB (AKT) pathway. Tremblay's group also showed that the levels of ErbB3 and the phosphorylation of PKB (Ser473) were reduced in PTP1B null tumors. Furthermore, loss of PTP1B correlated with reduced levels of cyclin D1. As might be anticipated, these effects on Ras/MAP kinase and PI3 kinase/PKB signaling induced by loss of PTP1B coincided both with decreased proliferation and increased apoptosis in the tumors. Perhaps most striking was the observation that administration of an orally bioavailable small-molecule inhibitor of PTP1B to the NDL2 mice also delayed tumorigenesis. Inhibition of PTP1B was demonstrated by the lowering of blood glucose to levels comparable to those in NDL2-*ptp1B^{-/-}* animals. Overall, these studies lead to the same conclusion—PTP1B is an important positive regulator of ErbB2 signaling, and inhibition of PTP1B function attenuates mammary tumorigenesis and malignancy.

PTP1B is localized on the cytoplasmic face of endoplasmic reticulum membranes and has been implicated in the dephosphorylation and inactivation of RPTKs that have undergone endocytosis (Haj et al., 2002). It has been suggested that ErbB2 undergoes slow endocytosis, emphasizing the importance of signaling events initiated at the plasma membrane. This raises the question of how an ER-targeted PTP can exert a positive effect on ErbB2-

induced signaling. Nevertheless, it is now clear that RPTKs remain active after internalization on endosomes, and Ras/MAP kinase signaling can be initiated from internal membranes (Mor and Philips, 2006). Whether the positive role of PTP1B demonstrated in these studies is due to regulation of ErbB2 signaling initiated at the endomembranes or due to regulation of complementary signaling pathways that synergize with plasma membrane-bound ErbB2 remains to be determined. Therefore, it will be important not only to define all the critical substrates of PTP1B in this context, but also to establish where such dephosphorylation events are taking place within the cell. Although there appears to be a cell-autonomous component to the effects of PTP1B deficiency on ErbB2 signaling, it will be important also to assess the contribution of non-cell-autonomous effects on tumorigenesis, such as potential disruption of immune cell function in the PTP1B knockout animals.

It is now clear that the PTPs do not simply function as passive antagonists of PTKs, but rather their activity is coordinated with that of the kinases, such that they are critical regulators of signaling in their own right. Recently, the positive role of PTPs in signaling was highlighted by the identification of *ptpN11* (SHP2) as the first PTP oncogene, which is mutated in several leukemias (Mohi and Neel, 2007). As described here, PTP1B has now been shown to play a positive role in regulating signaling events associated with breast tumorigenesis. In fact, Tremblay's group demonstrated that MMTV-directed overexpression of PTP1B alone was sufficient to drive mammary tumorigenesis (Julien et al., 2007). Such observations highlight the potential importance of PTPs themselves as therapeutic targets in cancer. Many major drug discovery efforts have been initiated in the pharmaceutical industry to focus on PTP1B as a therapeutic target for diabetes and obesity. These have generated extremely potent and selective inhibitors that may prove

effective in a cancer context. The studies highlighted here suggest the exciting possibility that combination therapies targeting both ErbB2 and PTP1B may offer new avenues for the treatment of breast cancer.

REFERENCES

- Betires-Alj, M., and Neel, B.G. (2007). *Cancer Res.* 67, 2420–2424.
- Bjorge, J.D., Pang, A., and Fujita, D.J. (2000). *J. Biol. Chem.* 275, 41439–41446.
- Dube, N., Cheng, A., and Tremblay, M.L. (2004). *Proc. Natl. Acad. Sci. USA* 101, 1834–1839.
- Haj, F.G., Verveer, P.J., Squire, A., Neel, B.G., and Bastiaens, P.I. (2002). *Science* 295, 1708–1711.
- Hermiston, M.L., Xu, Z., and Weiss, A. (2003). *Annu. Rev. Immunol.* 21, 107–137.
- Hynes, N.E., and Lane, H.A. (2005). *Nat. Rev. Cancer* 5, 341–354.
- Julien, S.G., Dube, N., Read, M., Penney, J., Paquet, M., Han, Y., Kennedy, B.P., Muller, W.J., and Tremblay, M.L. (2007). *Nat. Genet.* Published online January 28, 2007. 10.1038/ng1963.
- Kaminski, R., Zagodzon, R., Fu, Y., Mroz, P., Fu, W., Seng, S., Avraham, S., and Avraham, H.K. (2006). *Cancer Res.* 66, 5757–5762.
- Mohi, M.G., and Neel, B.G. (2007). *Curr. Opin. Genet. Dev.* 17, 23–30.
- Mor, A., and Philips, M.R. (2006). *Annu. Rev. Immunol.* 24, 771–800.
- Tonks, N.K. (2003). *FEBS Lett.* 546, 140–148.