Protein-Tyrosine Phosphatase 1B Is Required for HER2/Neu–Induced Breast Cancer

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Abstract

The protein-tyrosine phosphatase 1B (PTP1B; PTPN1) is an important regulator of mammalian metabolism and also helps control signaling by growth factors, cytokines, and extracellular matrix. Gene knockout studies in mice established PTP1B as a key negative regulator of the insulin and leptin receptors. Experiments using PTP1B−/− fibroblast lines, dominant-negative mutants, or small interfering RNAs indicate that PTP1B contributes to dephosphorylation of the epidermal growth factor receptor and platelet-derived growth factor receptors as well. However, PTP1B also may have some positive (signal enhancing) roles downstream of some growth factor receptors and integrins. Previous studies indicated that PTP1B is overexpressed in a significant subset of breast and ovarian cancers, especially in those overexpressing HER2/Neu (HER2+ tumors). However, experiments using tissue culture cells yield conflicting results on the effects of PTP1B in HER2 signaling, leaving the consequences of PTP1B overexpression for breast carcinogenesis unclear. To determine how PTP1B deficiency affects HER2-evoked breast tumorigenesis, we generated mouse mammary tumor virus (MMTV)–NeuNT transgenic mice lacking one or both alleles of PTP1B. Although heterozygous loss of PTP1B has no effect on tumorigenesis, homozygous PTP1B deficiency dramatically delays or prevents the onset of MMTV-NeuNT–evoked breast tumors. The effects of PTP1B deficiency correlate with defective extracellular signal-regulated kinase activation in preneoplastic mammary glands from compound mutant mice. In contrast, PTP1B deficiency has no effect on MMTV-polymyota middle T tumorigenesis. Our data raise the possibility that PTP1B inhibitors may be chemopreventive for some forms of breast cancer.


Introduction

The receptor tyrosine kinase HER2 (Neu/ErbB2) is amplified and overexpressed in ~25% of human breast cancer and plays a causal role in mammary carcinogenesis. HER2 overexpression is an adverse prognostic feature in early-stage breast cancer, associated with high tumor grade, increased tumor size, and increased nodal metastases (1). Transgenic overexpression of an activated form of HER2 (NeuNT) in the mouse mammary gland, under the control of mouse mammary tumor virus (MMTV) promoter (MMTV-NeuNT mice), causes glandular hyperplasia followed by mammary adenocarcinoma (2). The strongest evidence that HER2 overexpression plays a critical role in human breast cancer is the therapeutic efficacy of the anti-HER2 monoclonal antibody trastuzumab (Herceptin; ref. 3).

Tyrosyl phosphorylation is a reversible reaction, mediated not only by protein-tyrosine kinases, such as HER2, but also by protein-tyrosine phosphatase (PTP; ref. 4). Like the protein-tyrosine kinases, PTPs comprise a large superfamily, yet little is known about the roles of specific PTPs in the normal mammary gland or in breast carcinogenesis. The PTPN1 gene, encoding PTP1B, is located within 20q13, a region frequently amplified in ovarian and breast cancers and usually associated with a poor prognosis (5). Previous immunocytochemical studies showed that PTP1B is overexpressed in a significant subset (72%) of human breast (6) and ovarian (7) tumors and noted a correlation between PTP1B and HER2/Neu overexpression (6). Furthermore, PTP1B expression reportedly is elevated in HER2/Neu–transformed human breast epithelial cells and tumors derived from such cells (8).

PTP1B is a nonreceptor PTP localized on the cytoplasmic surface of the endoplasmic reticulum (9). Biochemical and genetic studies have established that PTP1B plays a critical role in regulating body mass and glucose homeostasis, by acting as a key insulin receptor and leptin receptor phosphatase. PTP1B also is required for normal rates of epidermal growth factor receptor and platelet-derived growth factor receptor dephosphorylation in fibroblasts, although notably, PTP1B−/− mice show no obvious evidence of increased epidermal growth factor receptor or platelet-derived growth factor receptor activity (e.g., tumors and fibrosis; refs. 10–12). In addition to its effects on receptor tyrosine kinase signaling, PTP1B can dephosphorylate the inhibitory tyrosyl phosphorylation site on Sre family kinases in human breast cancer cell lines or in response to integrin signaling in immortalized fibroblasts and platelets, thereby promoting Sre family kinase activation (9, 13). PTP1B also is proposed to dephosphorylate the scaffolding adapter p62DOK, which binds p120 Ras GTase-activating protein (RasGap) and promotes Ras/extracellular signal-regulated kinase (Erk) pathway inactivation. In the absence of PTP1B, p120 RasGap expression is increased and p62DOK phosphorylation is enhanced, resulting in lower levels of Ras/Erk activation in some lines of immortalized mouse embryo fibroblasts from PTP1B−/− mice (14). Biochemical studies have also implicated PTP1B as a signal transducer and activator of transcription 5 phosphatase in prolactin signaling (9).

Sre family kinase, signal transducer and activator of transcription, and the Ras/Erk pathway are major components of HER2/Neu signaling, making it difficult to predict the net effect of PTP1B overexpression on HER2-induced oncogenesis (1, 3). Indeed, PTP1B expression results in complex effects, some positive and some negative, on transformation of fibroblasts. For example, PTP1B-overexpressing fibroblasts are resistant to transformation by HER2 in ex vivo assays and have variable tumorigenic properties (15),
whereas PTP1B inhibits fibroblast transformation by v-Crk, v-Src, and v-Ras but not v-Raf (16).

Many pharmaceutical companies are attempting to develop small-molecule or antisense inhibitors of PTP1B for the treatment of metabolic disorders. Consequently, it is important to assess potentially deleterious effects of PTP1B inhibition (e.g., increased tumor generation). To determine how PTP1B affects HER2-evoked mammary tumorigenesis, we analyzed compound MMTV-NeuNT/PTP1B-deficient mice.

Materials and Methods

Transgenic mice. MMTV-NeuNT (strain TG.NK) and MMTV-PyMT mice were purchased from Charles River (Wilmington, MA) and The Jackson Laboratory (Bar Harbor, ME), respectively. PTP1B+/C0/C0 mice (129Sv/C2C57B6/J) were described previously (10). Females obtained from PTP1B+/C0/C0NeuNT (or PyMT)/PTP1B+/C0 mice, containing one copy of NeuNT or (PyMT), were kept as virgins for the entire period of the study. Mice were monitored for tumors by palpation twice weekly. All animal studies were approved by the Harvard Medical Area Standing Committee on Animals.

Reagents. Rabbit polyclonal anti-mouse PTP1B antibodies were described previously (10). Mouse monoclonal anti-FAK was a gift of Dr. Shiela Thomas (Harvard Medical School, Boston, MA). Commercial antibodies included the following: rabbit polyclonal anti-HER2, anti-pAkt473, anti-pSrc416, non-pSrc527, and anti–phospho-Erk (Cell Signaling, Danvers, MA); rabbit polyclonal anti-Shp2, mouse monoclonal Erk2, and RasGap (Santa Cruz Biotechnology, Santa Cruz, CA); and rabbit polyclonal anti-pFAK (Biosource, Camarillo, CA).

Protein analysis. Mammary tissues and tumors were lysed in a modified radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl (pH 7.5); 1% NP40; 150 mmol/L NaCl; 0.5% sodium deoxycholate; 0.1% SDS; 5 mmol/L EGTA; 2 mmol/L phenylmethylsulfonyl fluoride; 2 μg/mL each of aprotinin, leupeptin, and pepstatin; 2 mmol/L Na3VO4; 10 mmol/L NaF; 10 mmol/L...
that PTP1B is required for Erk activation in immortalized fibroblasts via two mechanisms: dephosphorylation of p62DOK and regulation of p120RasGap expression via a negative feedback pathway (14). Notably, p120RasGap expression in preneoplastic glands was not affected by PTP1B status (Fig. 2C). We attempted to analyze p62DOK tyrosyl phosphorylation on immunoprecipitates from mammary gland lysates from MMTV-NeuNT: PTP1B+/+ and MMTV-NeuNT: PTP1B−/− mice but were unable to obtain consistent results using commercially available antibodies. Given these technical limitations, we cannot exclude the possibility that increased p62DOK phosphorylation (and consequently increased RasGap recruitment) accounts for decreased Erk activation in preneoplastic PTP1B−/− mammary glands, although at this point, the detailed mechanism for diminished Erk activation remains unclear. We also compared mammary tumors from MMTV-NeuNT: PTP1B+/+ and MMTV-NeuNT: PTP1B−/− mice. In contrast to the preneoplastic gland, we observed no consistent differences in Erk activation in tumor lysates (Fig. 3A). These findings suggest that MMTV-NeuNT: PTP1B−/− mice that develop mammary carcinoma must circumvent the PTP1B requirement for Erk activation and activate the Erk pathway by different means. Akt, Src, FAK, and signal transducer and activator of transcription 5 phosphorylation and RasGap and HER2 levels in mammary tumors also were unaffected by PTP1B status (Fig. 3A–D; data not shown).

Our data suggest that PTP1B activity is required in preneoplastic MMTV-NeuNT mammary tissue, most likely to promote effective Erk activation. Interestingly, we showed recently that another PTP, Shp2, also is required for normal Erk activation in HER2-mediated carcinogenesis, where it acts downstream of the scaffolding adapter Gab2 (17). The requirement for two PTPs as positive regulators of the Erk pathway in HER2-mediated carcinogenesis emphasizes the need for exquisite control of this pathway and further shows the diverse contributions made by PTP family members to receptor tyrosine kinase signaling. PTP1B is expressed ubiquitously; therefore, at least some of the effects of PTP1B deficiency on mammary carcinogenesis could be non-cell autonomous. For example, recent studies have emphasized the complex
roles of cells of the immune system in both promoting and protecting against cancer (18, 19). PTP1B−/− mice exhibit altered macrophage function, an accumulation of B cells in bone marrow and lymph nodes and an increased percentage of B cells in blood, which could contribute to altered HER2-evoked tumor incidence (20, 21). In the regard, PTP1B−/− mammary tumors seemed to contain much higher levels of immunoglobulin than those from WT mice. Alternatively, the lower insulin levels in PTP1B−/− mice could impair tumorigenesis. Although the lower levels of Erk activation in preneoplastic mammary tissue from PTP1B−/− (compared with WT) mice suggest at least some cell autonomous effects of PTP1B deficiency on HER2-evoked tumorigenesis, analysis of mice lacking PTP1B specifically in the epithelial versus the stromal compartments are needed to resolve this issue unambiguously. Such studies will be facilitated by our recent generation of inducible (floxed) PTP1B knockout mice (12).

PTP1B knockout mice are insulin and leptin hypersensitive and are protected against diet-induced obesity, insulin resistance, and diabetes (10–12). Several pharmaceutical companies have programs to develop PTP1B inhibitors to address these medically important disorders. Because such agents would have to be taken for long periods, they require a high degree of safety. Given the pleiotropic effects of PTP1B on receptor tyrosine kinase signaling and the role of dysregulated receptor tyrosine kinase signaling in human cancer, the possibility that PTP1B inhibitors might increase cancer incidence has been a serious concern (9). Indeed, PTP1B deficiency increases the susceptibility to the development of B lymphoma in mice lacking p53 (20) and increases IGF1-induced cell survival, plating efficiency, and cell motility in transformed cells (22). Our data suggest that, in contrast, PTP1B deficiency has no (MMTV-PyMT) or a protective role (MMTV-NeuNT) in breast oncogenesis. Consequently, the use of PTP1B inhibitors for metabolic disease may have beneficial effects in breast cancer chemoprevention.

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