Molecular Pathways: Targeting Protein Tyrosine Phosphatases in Cancer

Lakshmi Reddy Bollu, Abhijit Mazumdar, Michelle I. Savage, and Powel H. Brown

Abstract

The aberrant activation of oncogenic signaling pathways is a universal phenomenon in cancer and drives tumorigenesis and malignant transformation. This abnormal activation of signaling pathways in cancer is due to the altered expression of protein kinases and phosphatases. In response to extracellular signals, protein kinases activate downstream signaling pathways through a series of protein phosphorylation events, ultimately producing a signal response. Protein tyrosine phosphatases (PTP) are a family of enzymes that hydrolytically remove phosphate groups from proteins. Initially, PTPs were shown to act as tumor suppressors and oncogenic PTPs in cancer and discuss the current status of PTP inhibitors for cancer therapy.

PTPs belong to a superfamily of enzymes that hydrolytically remove phosphate groups from proteins (2). Genome sequencing studies have shown that the human genome includes more than 100 genes that encode PTPs (3, 4). These enzymes function as holoenzymes and share a consensus sequence (known as the HCXnR motif, where X is any amino acid) in the active site of the enzyme (5). PTPs are classified on the basis of their cellular localization, specificity, and function. Cellular localization determines whether PTPs are grouped as receptor-type PTPs (PTPR), which are localized to the plasma membrane, or non-receptor-type PTPs (PTPN), which are localized in the cytosol. Similarly, based on their specificity, PTPs are grouped into either tyrosine-specific phosphatases or dual-specific phosphatases (which remove a phosphate group from tyrosine and/or serine/threonine residues).

PTPs are broadly classified into two groups based on their function: tumor suppressor PTPs or oncogenic PTPs. These tumor suppressor/oncogenic PTPs may belong to the other groups described above based on their cellular localization and specificity. The loss of tumor suppressor PTP function is often observed in cancer due to gene deletions, mutations, and epigenetic modifications, such as promoter methylation. Loss of these tumor suppressor PTPs leads to the hyperactivation of signaling pathways and promotes tumorigenesis. PTEN is the most frequently mutated tumor suppressor gene in human cancers and is overexpressed in triple-negative breast cancer (TNBC), and increases in vivo PI3K/Akt signaling and oncogene-induced transformation and tumor growth while reducing metastasis in mouse models (6).

Phosphatases as Tumor Suppressors

Several tumor suppressor PTPs have been identified whose loss has been shown to promote tumorigenesis, growth, and
metastasis in in vitro and in vivo models (Table 1). The first tumor suppressor phosphatase identified was PTEN, which was found to be frequently lost or mutated in many human cancers at high frequency (reviewed in refs. 7, 8). PTEN dephosphorylates phosphoinositide substrates, which leads to inhibition of the AKT/PKB kinase cascade. Mutation or loss of PTEN results in unopposed activation of AKT, leading to growth promotion and tumor development. Laboratory studies have shown that deletion of PTEN in normal cells promotes transformation of these cells (9) and that PTEN-deficient mice develop cancers (10).

More recently, other tumor suppressor phosphatases have been identified. These include DEP1 [also known as PTP receptor (PTPR) type J] that is frequently deleted or mutated in many human cancers, including breast and colon cancers (Table 1; ref. 11). Loss of DEP1 is associated with increased growth factor receptor signaling and promotes cell growth (12). Recently, Mazumdar and colleagues identified dual specificity phosphatase 4 (DUSP4) as the most commonly underexpressed protein phosphatase in estrogen receptor-negative (ER−) breast cancers, and showed that ectopic expression of DUSP4 inhibits the growth and invasive properties of ER− breast cancer cells by dephosphorylating growth-promoting signaling proteins (13). Similarly, loss of DUSP4 promotes the progression of intraepithelial neoplasms into invasive carcinoma in the pancreas (14). Moreover, loss of DUSP4 activates the activation of the MAPK pathway, thereby promoting a stem cell–like phenotype and dampening the response to neoadjuvant therapy in breast cancer (15, 16).

Further studies identified loss-of-function mutations in six other PTPs in several human cancers (including colorectal, lung, breast, and gastric cancer): PTPRF, PTPRM, and PTP non-receptor type (PTPN) 3 (PTPN3), PTPN13, and PTPN14 (Table 1; ref. 17). Expression of the wild-type form of these mutated genes suppresses the growth of colon cancer cells (17).

Sun and colleagues identified PTPN12 as a tumor suppressor through a genetic screen and showed that PTPN12 inactivation promotes the transformation of mammary epithelial cells through activation of the EGFR/HER2 signaling axis (Table 1; ref. 18). In addition to gene deletions and inactivating mutations, loss of tumor suppressor PTP function may be due to loss of expression by epigenetic modifications in the promoter region. Analysis of the methylation patterns of CpG islands within the promoter regions has revealed that loss of PTPRF, PTPRM, and PTPRK expression is due to hypermethylation in leukemia samples (19). Loss of PTPRK leads to hyperactivation of Akt signaling and promotes colon cancer progression (20). PTPRK also functions as a tumor suppressor by inhibiting the phosphorylation of EGFR (21) and STAT3, and subsequently inhibiting tumor growth and invasive ability (22).

Hypermethylation of promoter regions may suppress the expression of several other tumor suppressor phosphatases. Promoter methylation silences the expression of DUSP6 in primary pancreatic cancers (23) and PTPRO in hepatocellular carcinoma, lung cancer, and leukemia (Table 1; ref. 24). Overexpression of PTPRO induces antiproliferative/transformational and apoptotic properties in leukemia and lung cancer cells (25), and PTPRO promoter methylation is a predictive marker for poor prognosis in HER2− breast cancer patients (26). Hypermethylation of the SHP1 (PTPN6) promoter has been reported in leukemias/lymphomas, and treatment with a DNA methylation inhibitor reactivated SHP1 expression. Loss of SHP1 is associated with both clinical stage and pathogenesis of leukemia and lymphomas (27). DNA methylase I and STAT3 signaling mediate the silencing of SHP1 expression (28).

### Phosphatases as Oncogenes

Recent studies have demonstrated that PTPs can also function as oncogenes to promote tumor development (Table 2). The expression of these oncogenic PTPs is aberrantly elevated in

<table>
<thead>
<tr>
<th>PTP</th>
<th>Cancer</th>
<th>Expression</th>
<th>Altered signaling pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHP2 (PTPNII)</td>
<td>Breast, leukemia, and gliomas</td>
<td>Overexpression and activating mutations</td>
<td>EGFR/Ras/MAPK</td>
<td>29-35</td>
</tr>
<tr>
<td>PTP1B (PTPN1)</td>
<td>Ovarian, gastric, prostate, and breast</td>
<td>Overexpression</td>
<td>Src/Ras/Erk and PISK/Akt</td>
<td>36-43</td>
</tr>
<tr>
<td>PTP4A3 (PRL3)</td>
<td>Breast, gastric, and colon</td>
<td>Overexpression</td>
<td>PDGF, integrin, and EphA signaling</td>
<td>1, 44-46</td>
</tr>
</tbody>
</table>

Table 1. Tumor suppressor PTPs and cancer

<table>
<thead>
<tr>
<th>PTP</th>
<th>Cancer</th>
<th>Mechanism for reduced expression</th>
<th>Altered signaling pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>Prostate and breast</td>
<td>Mutations and deletions</td>
<td>PI3K</td>
<td>7-10</td>
</tr>
<tr>
<td>INP5J</td>
<td>Breast</td>
<td>Transcriptional repression</td>
<td>PI3K</td>
<td>2</td>
</tr>
<tr>
<td>SHP1</td>
<td>Leukemia and lymphomas</td>
<td>Methylation of promoter</td>
<td>JAK/STAT signaling</td>
<td>27, 28</td>
</tr>
<tr>
<td>DEP1</td>
<td>Breast and colon</td>
<td>Mutations and deletions</td>
<td>FLT3, Erk1/2, and Akt signaling</td>
<td>11, 12</td>
</tr>
<tr>
<td>PTPRF</td>
<td>Colon, breast, and lung</td>
<td>• Somatic mutations</td>
<td>PDGF, Akt, and Akt signaling</td>
<td>17</td>
</tr>
<tr>
<td>PTEN</td>
<td>Breast</td>
<td>• Deletions</td>
<td>JAK and integrin</td>
<td>17</td>
</tr>
<tr>
<td>DUSP4</td>
<td>Breast, pancreas, and thyroid</td>
<td>Copy number loss</td>
<td>STAT3 and axillin</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>Leukemia, lung, and breast</td>
<td>Methylation of promoter</td>
<td>EGFR/MAPK signaling</td>
<td></td>
</tr>
<tr>
<td>PTPN3</td>
<td>Breast</td>
<td>Mutations</td>
<td>Src signaling</td>
<td></td>
</tr>
<tr>
<td>PTPN4</td>
<td>Breast</td>
<td>Mutations</td>
<td>YAP signaling</td>
<td></td>
</tr>
<tr>
<td>PTPN12</td>
<td>Breast</td>
<td>Mutations</td>
<td>EGFR, HER2, and mTOR</td>
<td>18</td>
</tr>
<tr>
<td>PTPRK</td>
<td>Colon and lymphoma</td>
<td>Methylation of promoter</td>
<td>EGFR and Akt signaling</td>
<td>19, 20</td>
</tr>
<tr>
<td>DUSP6</td>
<td>Leukemia, lung, and promoter</td>
<td>Methylation of promoter</td>
<td>MAPK signaling</td>
<td>27</td>
</tr>
<tr>
<td>DUSP4</td>
<td>Breast, pancreas, and thyroid</td>
<td>Copy number loss</td>
<td>MAPK, JNK, RB, and NF-κB</td>
<td>13-16</td>
</tr>
<tr>
<td>PTPRO</td>
<td>Leukemia, lung, and breast</td>
<td>Methylation of promoter</td>
<td>PI3F/FOXMI</td>
<td>24-26</td>
</tr>
</tbody>
</table>

Table 2. Oncogenic PTPs and cancer
several human cancers, which lead to tumor formation, growth, and metastasis, ultimately leading to diminished survival.

Src-homology 2 (SH2) domain containing protein tyrosine phosphatase 2 (SHP2), also known as PTPN11, was the first oncogenic PTP to be reported (Table 2). Despite sharing homologous structure and sequence with the tumor suppressor SHP1 (PTPN6), SHP2 functions as an oncogene, and its overexpression has been reported in human leukemia (29) and breast cancers (30). Activating SHP2 somatic mutations were initially identified in patients with Noonan syndrome; however, these mutations are associated with childhood (but not adult) malignancies, such as chronic myelomonocytic leukemia (~35%) and acute myeloid leukemia (31). SHP2 contains two SH2 domains: a PTP catalytic domain, and a C-terminal domain with tyrosine phosphorylation sites and a proline-rich domain that mediate protein–protein interactions. SHP2 is a PTPN that, contrary to tumor suppressor PTPs, positively regulates cell signaling pathways activated by growth factors, cytokines, and hormones and promotes cell growth, motility, differentiation, and survival. Upon EGF stimulation, EGFR activates SHP2 through growth factor receptor bound protein 2 (GRB2)–associated binding protein 1 (GAB1; ref. 32), and several studies have demonstrated that activated SHP2 promotes the Ras/ERK pathway by dephosphorylating RasGAP, a negative regulator of Ras activation (33). A recent study by Bunda and colleagues demonstrates that SHP2 activates the Ras signaling pathway in glioma by dephosphorylating Ras at tyrosine residue 32, which blocks Ras–Raf binding (34). In addition, SHP2 inhibition reduces the growth and invasiveness of breast cancer cells by inhibiting the Ras/ERK and PI3K/Akt signaling pathways (35).

PTP1B, a non-receptor–type oncogenic PTP, also known as PTPN1, is involved in growth factor signaling (Table 2). The PTP1B gene is frequently amplified in ovarian, gastric, prostate, and breast cancers and correlates with poor prognosis (36–39). Knockdown of PTP1B reduces cell growth and induces both cell-cycle arrest and apoptosis. Knockdown of PTP1B reduces migration and invasion by reversing the epithelial–mesenchymal transition (EMT) process (40). PTP1B expression is elevated in HER2-transformed human breast epithelial cells and tumors. Knockout of PTP1B or use of a pharmacologic PTP1B inhibitor delays the onset of mammary tumor formation and reduces lung metastasis in the MMTV Her2/NeuT mouse model by reducing Ras/MAPK and Akt signaling (41). Furthermore, tissue-specific overexpression of PTP1B alone induces mammary tumorigenesis (41). Consistently, the elevated PTP1B expression promotes cell proliferation and metastasis in non–small cell lung cancer through the activation of the Src/Ras/ERK and PI3K/Akt signaling pathways (42). However, in contrast to its oncogenic role, PTP1B can also function as a tumor suppressor, as genetic depletion of PTP1B has been shown to accelerate lymphomagenesis in p53-null mice (43). PTP4A3, another oncogenic PTP (also known as PRL3), is highly expressed in breast, colon, gastric, and liver cancers and has been shown to play an important role in cell proliferation and metastasis (Table 2). Src-dependent phosphorylation of PTP4A3 promotes cell motility and invasion through the activation of Rho (44). In contrast, tyrosine phosphatase analysis has revealed that overexpression of PTP4A3 induces aberrant Src activation, suggesting that PTP4A3 works both downstream and upstream of Src (45). Furthermore, PDGF, integrin, and Eph receptors are key players in PTP4A3–overexpressing cells (45). PTP4A3 directly binds integrin-beta 1, suggesting an involvement in focal adhesion signaling.

den Hollander and colleagues (2016) explored the expression levels of PTPs in 102 human breast cancer samples to identify PTPs that are differentially overexpressed in TNBC. In this study, 146 PTPs were differentially expressed in TNBC as compared with ER+ breast cancer samples. Nineteen of these PTPs were significantly upregulated and 27 were downregulated in TNBC. Of these 19 upregulated PTPs, the growth of triple-negative compared with ER+ breast cancer cells was significantly reduced with the knockdown of six PTPs: PTP4A3, phospholipid phosphatase 3 (PAP2B), discs large homolog associated protein 5 (DLGAP5), cell division cycle 25B (CDC25B), phosphoserine phosphatase (PSPH), and translocase of inner mitochondrial membrane 50 (TIMM50; Table 2). Further screening of these PTPs through anchorage-independent growth assays and cell-cycle progression analyses demonstrated that knockdown of PTP4A3 was found to have the greatest suppression of cell growth. In addition, analysis of publicly available breast cancer datasets revealed that PTP4A3 expression is significantly elevated in TNBC samples, and in silico analysis revealed that increased PTP4A3 expression is due to gene amplification in 29% of basal-like breast cancers. Depletion of PTP4A3 reduced TNBC tumor growth in vivo by reducing proliferation marker (Ki67), phospho-ERK1/2, and p38 levels while increasing apoptotic markers (1). Highly elevated PTP4A3 expression is also associated with enhanced tumor formation and metastasis by reducing tumor-initiating cells in colon cancer mouse models (46). Collectively, these studies demonstrate that oncogenic PTPs play a critical role in tumor initiation, progression, and metastasis by enhancing signaling pathways and serving as valuable targets for cancer therapy.

Clinical–Translational Advances

It is now clear that several types of human cancer are associated with abnormal PTP signaling. PTPs fall into two broad groups: tumor suppressing and oncogenic phosphatases, with tumor suppressor PTP expression lost in cancers and oncogenic PTP expression elevated in cancers. Increasing evidence highlights the physiologic importance of PTPs in tumor initiation, growth, and invasiveness and has consequently fueled interest in developing PTP-targeted therapies for treating cancer. The concept of targeting PTPs for human diseases initially arose from the discovery that genetic depletion of PTP1B increases insulin sensitivity and resistance to high fat diet–induced obesity (47).

As the disruption and/or loss of tumor suppressor PTP function in cancer is due to gene deletions, mutations, or epigenetic modifications, it is not feasible to reactivate the function or expression of tumor suppressor PTPs that are deleted at both loci. Somatic mutations inactivate six of the PTPs in colon cancer, and there are no drugs currently available that reactivate the wild-type function of these mutant PTPs. The last subset of tumor suppressor PTPs is silenced due to epigenetic modifications (e.g., hypermethylation of the promotor region); however, the specific DNA methylases and/or methyltransferases must be identified to develop DNA methylation inhibitors capable of reactivating PTP expression and thereby serving as potential agents for cancer therapy. To date, the FDA has approved one DNA methylation inhibitor, 5-Aza-cytidine (nucleoside analogue), for the treatment of hematologic malignancies. The expansion of the application of this drug to other cancers with epigenetically silenced tumor
suppressor PTPs could greatly impact future cancer therapy and possibly cancer prevention.

Overexpression of oncogenic PTPs has been shown to be critical for tumor development, growth, and metastasis, as evidenced by reduced tumor formation, growth, and lung metastasis in PTP1B knockout MMTV-Her2 mice (39). Similarly, several other studies have demonstrated the importance of oncogenic PTPs, including SHP2 and PTP4A3, in a variety of tumor types, as discussed above.

Inhibitors of oncogenic phosphatases

Although oncogenic PTPs are attractive molecules for the development of targeted therapies, the development of PTP inhibitors remains challenging. Selectivity poses a major challenge in developing PTP inhibitors due to the high level of conservation in the active site of both tumor suppressor and oncogenic PTP subtypes. Thus, the inhibitors that target the active sites of oncogenic PTPs also inhibit tumor suppressor PTPs in normal healthy tissues of patients and potentially result in adverse side effects. However, these adverse side effects could be resolved by designing selective PTP inhibitors that bind to regions located externally to the PTP active site.

The oncogenic role of PTP1B has been well characterized using both in vitro and in vivo models for various human cancers. In 2003, Xie and colleagues reported the first potent PTP1B inhibitor (48). Using a second nonconserved aryl phosphate–binding site adjacent to the active site of PTP1B, Xie and colleagues developed bidentate ligands as PTP1B inhibitors. These bidentate ligands bind to both the active site and a unique adjacent aryl phosphate–binding site. These bidentate PTP1B inhibitors are the most potent inhibitors of PTP1B to date ($K_i$ value, 2.4 nmol/L), providing as much as 1,000- to 10,000-fold selectivity for PTP1B as compared with other PTPs; however, the initial PTP1B inhibitor did not affect cancer cell growth due to a lack of cell permeability properties (48). Therefore, this bidentate ligand was modified with a fatty acid moiety, thereby increasing its cell membrane permeability; the resulting analogue of this compound is highly active in blocking PTP1B and shows remarkable antigrowth effects in several cancer cell lines (49). A number of additional PTP1B inhibitors have also been developed in recent years. Scapin and colleagues developed benzotriazole inhibitors of PTP1B with an IC$_{50}$ of 5 nmol/L; unfortunately, these inhibitors had limited cell permeability (50). Another compound, difluoromethyl phosphonate, developed at Merck, has been shown...
data obtained from ClinicalTrials.gov.

Table 3. PTP inhibitors and clinical trials

<table>
<thead>
<tr>
<th>PTP inhibitor</th>
<th>Sponsor</th>
<th>CT identifier</th>
<th>Disease</th>
<th>Clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trodusquemine</td>
<td>PTPIb</td>
<td>NCT00606112</td>
<td>Type II diabetes and obesity</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>MSI-1436C</td>
<td>PTPIb</td>
<td>NCT02524951</td>
<td>Metastatic breast cancers</td>
<td>Phase I (recruiting)</td>
</tr>
<tr>
<td>SSG</td>
<td>SHP2</td>
<td>NCT00662012</td>
<td>Leishmaniasis</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>SSG</td>
<td>SHP2</td>
<td>NCT00498979</td>
<td>Stage IV melanoma</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>SSG</td>
<td>SHP2</td>
<td>NCT00629200</td>
<td>Advanced solid tumors</td>
<td>Phase I completed</td>
</tr>
</tbody>
</table>

Future of Phosphatase Inhibitors for Cancer Therapy

Recent research has demonstrated that oncogenic PTPs are attractive candidates for the development of targeted thera-
pies for multiple cancer types. However, the development of specific PTP inhibitors has been challenging, resulting in a lack of effective and safe PTP inhibitors for clinical use. The results of a phase I trial with the SHP2 inhibitor SSG showed that patients with advanced solid tumors and stage IV melanoma well tolerated the concentrations of 900 and 1,200 mg/m², respectively (59, 60). Similarly, positive results from the ongoing phase I clinical trial of the PTP1B inhibitor MSI-1436C, defining its safety and tolerability in treating metastatic breast cancer patients, will be instrumental to the translation of this drug to the clinical setting. Although several additional phosphatases have also been shown to be attractive targets for cancer therapy, further research is required to identify specific critical sites located outside the active sites to successfully develop highly specific PTP inhibitors for cancer therapy.

Disclosure of Potential Conflicts of Interest

P.H. Brown is a consultant/advisory board member for Susan G. Komen Foundation. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: L.R. Bollu, A. Mazumdar, P.H. Brown
Development of methodology: A. Mazumdar, P.H. Brown
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Mazumdar
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.H. Brown
Writing, review, and/or revision of the manuscript: L.R. Bollu, M.I. Savage, P.H. Brown
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.I. Savage, P.H. Brown
Study supervision: P.H. Brown

Acknowledgments

We would like to thank Sam Short for her assistance with this submission.
Grant Support
This work was funded by a Susan G. Komen Promise Grant (KGB81694; to P.H. Brown), a Komen SAB grant (to P.H. Brown), a Breast Cancer Research Foundation grant (to P.H. Brown), a Norman Brinker Award for Research Excellence (to P.H. Brown), a John Charles Cain Distinguished Chair Award (to P.H. Brown), a Mariam Rogers Fund for Breast/Women’s Cancer Research (to P.H. Brown), a John F. & Julie Young Award (to P.H. Brown), and a Cancer Center Support Grant (S P40 CA016672-38).

Received September 26, 2016; revised November 7, 2016; accepted November 8, 2016. Published OnlineFirst January 13, 2017.

References


