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Targeting PTPs with small molecule inhibitors in cancer treatment

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Abstract

Protein tyrosine phosphorylation plays a major role in cellular signaling. The level of tyrosine phosphorylation is controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Disturbance of the normal balance between PTK and PTP activity results in aberrant tyrosine phosphorylation, which has been linked to the etiology of several human diseases, including cancer. A number of PTPs have been implicated in oncogenesis and tumor progression and therefore are potential drug targets for cancer chemotherapy. These include PTP1B, which may augment signaling downstream of HER2/*Neu*; SHP2, which is the first oncogene in the PTP superfamily and is essential for growth factor-mediated signaling; the Cdc25 phosphatases, which are positive regulators of cell cycle progression; and the PRL phosphatases, which promote tumor metastases. As PTPs have emerged as drug targets for cancer, a number of strategies are currently being explored for the identification of various classes of PTP inhibitors. These efforts have resulted many potent, and in some cases selective, inhibitors for PTP1B, SHP2, Cdc25 and PRL phosphatases. Structural information derived from these compounds serves as a solid foundation upon which novel anti-cancer agents targeted to these PTPs can be developed.

Keywords

Protein tyrosine phosphatase (PTP); PTP1B; SHP2; Cdc25; PRL phosphatases; small molecule inhibitor design; PTP inhibitor

1 Introduction

Protein tyrosine phosphorylation plays a major role in a vast array of cellular functions, including proliferation, differentiation, survival/apoptosis, and motility. The level of protein tyrosine phosphorylation is controlled by two opposing chemical reactions catalyzed by the protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP). Major insights into protein tyrosine phosphorylation mediated cellular signaling have been derived from studies of PTKs and it is common to view signaling pathways as cascades of reactions emanating from the PTKs [1]. Indeed, many transmembrane receptors for peptide hormones and growth factors possess intrinsic PTK activity. Receptors for cytokines lack intrinsic kinase activity but associate with non-receptor PTKs inside the cell. Recent studies indicate that the PTPs function collaboratively with the PTKs in modulating signaling pathways that a broad spectrum of physiological processes [2]. In fact, PTPs constitute a large family of enzymes (>100) that parallel PTKs in their structural diversity and complexity [3]. The hallmark that defines the PTP superfamily is the active site amino acid sequence (H/V)C(X)₅R(S/T), also called the PTP signature motif, in the catalytic domain.

Disturbance of the normal balance between PTK and PTP activity results in aberrant tyrosine phosphorylation, which has been linked to the etiology of several human diseases, including cancer [4-7]. For example, abnormal expression and activation of many receptor tyrosine

kinases, such as the epidermal growth factor receptor, and the proto-oncogene nonreceptor tyrosine kinases Src and Abl, are causative factors in cancer [8]. Consequently, PTPs are often viewed to reverse the PTK action and therefore sometime considered as products of tumor suppressor genes. Indeed, *PTEN*, the first tumor suppressor gene encoding a phosphatase in the PTP superfamily, is mutated (inactive) in several major types of neoplasia, including brain, breast, and prostate cancers [9,10]. Recent evidence suggest that, several PTPs, including RPTP ρ , RPTP γ , LAR, PTPH1, PTP-BAS, and PTPD2, may function as tumor suppressors and are frequently mutated in colon cancer [11].

Interestingly, PTPs can also potentiate, rather than antagonize, actions of PTKs. This mode of synergy enhances mitogenic signaling, leading to cell transformation. Thus ectopic expression of PTP α results in Src activation and causes transformation [12]. The mechanism for the PTP α induced Src activation is dephosphorylation of the inhibitory Tyr527 in the C-terminal tail of Src. Conversely, deletion of PTP α in mice leads to diminished kinase activity of Src which strongly suggests that PTP α is a physiological positive regulator of Src kinase in vivo [13,14]. SHP2 has been shown to play a positive role in a number of growth factor mediated signaling pathways and the phosphatase activity of SHP2 is required for the Ras-dependent cellular proliferation and survival [15-18]. A number of activating (gain of function) mutations in SHP2 have been identified as the cause of the inherited disorder Noonan syndrome [19] and several forms of leukemia and solid tumors [20,21]. Most recently, the PRL (phosphatase of regenerating liver) phosphatases have been implicated as potential oncogenes that promote cell growth and tumor invasion [22].

As discussed above, it appears that cellular pathways regulated by tyrosine phosphorylation could offer a rich source of drug targets for developing novel therapeutics [4,7,23]. The development of small molecule inhibitors to modulate protein tyrosine phosphorylation has transformed the approach to cancer therapy and is likely to have significant impact on other therapeutic areas. Indeed, small molecule inhibitors that block the activity of a narrow spectrum of PTKs exhibit much less toxicity than the currently used chemotherapeutic agents. The potential of such targeted therapeutics has been well demonstrated by the successful treatment of chronic myelogenous leukemia with imatinib mesylate [24] and non-small cell lung cancers with gefitinib [25], which target Bcr/Abl and epidermal growth factor receptor (ErbB1) aberrantly activated in the malignancies, respectively. Given the critical role of PTPs in cellular signaling and the fact that deregulation of PTP activity also contributes to the pathogenesis of a number of cancers [6,26], inhibitors of the PTPs are also expected to have therapeutic value. Furthermore, since no formal functional linkage exists between the PTKs and PTPs, i.e. a PTP may catalyze the dephosphorylation of substrate proteins phosphorylated by more than one PTK, inhibitors of the PTPs may also have unique modes of action. Thus, PTPs represent novel and attractive targets for cancer treatment. In the following, we summarize the major findings that establish PTP1B, Cdc25, SHP2, and PRL phosphatases as potential targets for the treatment of cancer, and highlight recent development in small molecule inhibitors targeted to these PTPs.

2 PTP1B

PTP1B is a ubiquitously expressed phosphatase that appears to be involved in the regulation of several growth factor signaling pathways. Biochemical and genetic studies have established that PTP1B plays a critical role in regulating body weight, glucose homeostasis, and energy expenditure by acting as a key negative regulator of insulin receptor and leptin receptor mediated signaling pathways. PTP1B-deficient mice display increased sensitivity to insulin, have improved glycemic control, and are more resistant to diet-induced obesity than wild-type mice [27,28]. Diabetic mice treated intraperitoneally with PTP1B antisense oligonucleotides have lower PTP1B protein levels in liver, leading to decreases in fat, plasma insulin, and blood

glucose levels [29]. These findings indicate that inhibition of PTP1B is an effective strategy for treating metabolic diseases such as type 2 diabetes and obesity.

Besides a role in insulin and leptin signaling, PTP1B may also be involved in several other physiological and pathological processes. For example, PTP1B is capable of catalyzing the dephosphorylation of activated epidermal growth factor receptor and platelet-derived growth factor receptor in fibroblasts [30,31], although PTP1B^{-/-} mice show no obvious evidence of increased epidermal growth factor receptor or platelet-derived growth factor receptor activity [27,28]. In addition to its effects on receptor tyrosine kinase itself, PTP1B may play positive (signal enhancing) roles downstream of growth factor receptors and integrins. Thus, PTP1B can remove the inhibitory tyrosyl phosphorylation site on Src family kinases in human breast cancer cell lines or in response to integrin signaling in fibroblasts, thereby promoting Src family kinase activation [32-34]. PTP1B has also been reported to dephosphorylate the scaffolding adapter protein p62^{DOK}, which binds and stimulates p120RasGap activity, resulting in activation of the Ras-ERK pathway [35]. Since PTP1B can promote both the Src kinase and the Ras/Erk pathways, which constitute major components of HER2/*Neu* signaling [36,37], PTP1B may represent a new therapeutic target in breast cancer. Interestingly, PTP1B is up-regulated in HER2/*Neu*-transformed cell lines [38] and 90% of all breast tumors overexpress both HER2/*Neu* and PTP1B [39]. Two recent genetic studies revealed that PTP1B is required for HER2/*Neu*-induced breast cancer and that PTP1B deficiency delays HER2/*Neu*-induced mammary tumorigenesis and protects from lung metastasis [40,41]. Moreover, PTP1B has also been shown to contribute to the oncogenic properties of colon cancer cells through Src activation [42]. These findings raise the possibility that selective inhibition of PTP1B may be beneficial for the treatment of breast and colon cancer.

Because of the well-established negative role of PTP1B in both insulin and leptin signaling, major efforts have been initiated from both pharmaceutical industry and academia to focus on PTP1B as a therapeutic target for diabetes and obesity. A key challenge facing PTP inhibitor development is the issue of selectivity due to the highly conserved nature of the PTP active site (i.e. pTyr binding site). The most productive approach to address this issue has been the design of bidentate ligands to engage both the PTP active site and a unique adjacent peripheral site for enhanced affinity and specificity. This usually entails the attachment of diversity elements to a properly functionalized nonhydrolyzable pTyr surrogate. These efforts have generated many highly potent and selective PTP1B inhibitors with varying degrees of in vivo efficacy [43]. Figure 1 highlights some of the most potent and selective PTP1B inhibitors that have been disclosed. Compound **I** identified from a combinatorial chemistry approach, displays a K_i value of 2.4 nM for PTP1B and exhibits several orders of magnitude selectivity in favor of PTP1B against a panel of PTPs [44]. Biochemical and structural analyses show that compound **I** simultaneously occupies both the active site and a unique peripheral site in PTP1B [45]. Unfortunately, compound **I** contains five negative charges at neutral pHs and is not cell permeable. In order to determine the effect of small molecule PTP1B inhibitor in vivo, a number of strategies have been employed to facilitate cellular uptake of compound **I**. Cell permeable derivatives of compound **I** were prepared by coupling it to either a highly lipophilic fatty acid (compound **II** in Figure 1) [46] or to the cell penetrating peptide (D)Arg₈ via a disulfide bridge (**III** in Figure 1) [47]. Moreover, a novel strategy was utilized to synthesize the prodrug compound **IV** (Figure 1) for cellular delivery of the phosphonate-based compound **I** [48]. Studies with these cell permeable compounds demonstrate that small molecule inhibitors of PTP1B can work synergistically with insulin to increase insulin signaling and augment the insulin stimulated glucose uptake [46-48]. Furthermore, pretreatment of leptin-resistant rats with compound **II** results in a marked improvement in leptin-dependent suppression of food intake [49].

A more recent example in PTP1B inhibitor development involves a rational design effort that resulted in the discovery of the 1,2,5-thiadiazolidin-3-one-1,1-dioxide group as a novel mimic the phosphoryl moiety of pTyr [50]. When incorporated into a di-peptide structure, the isothiazolidinone containing inhibitor **V** has a K_i of 0.19 μ M [51]. Using the isothiazolidinone group as the pTyr mimetic, a peptide-based inhibitor **VI** was synthesized which showed an IC_{50} of 40 nM, demonstrating the utility of the isothiazolidinone to serve as a highly efficacious pTyr mimetic [52]. To improve the cell permeability and oral bioavailability, a series of non-peptide based inhibitors using the same isothiazolidinone group as the pTyr mimetic were synthesized. Among them, compound **VII** displayed high inhibition potency, with an IC_{50} of 35 nM. It also exhibited significant cellular activity, increasing the insulin receptor phosphorylation level at 80 μ M [53]. Collectively, these results illustrate that it is highly feasible to obtain potent and selective PTP1B inhibitors. Pharmacological studies with these small molecule inhibitors have enabled important validation of PTP1B as a therapeutic target for metabolic disorders. Future studies of the effects of PTP1B inhibition on HER2-evoked mammary tumorigenesis and breast cancer chemoprevention may offer new avenues for the treatment of breast cancer with PTP1B inhibitors.

3 SHP2

SHP2 contains two Src homology-2 (SH2) domains, a PTP domain, and a carboxyl-terminal tail. In resting cells, SHP2 is autoinhibited through intramolecular noncovalent interactions between its N-SH2 domain and the PTP active site [54]. Upon growth factor or cytokine stimulation, the SH2 domains of SHP2 binds to tyrosine phosphorylated docking proteins such as Gab1 and Gab2, which releases the autoinhibition and activates the SHP2 phosphatase activity [55]. Activated SHP2 is essential for the Ras-dependent signaling and for the growth factor-stimulated cell proliferation [15-18]. Activating somatic mutations in *PTPN11* (which encodes SHP2) have been found in patients with Noonan syndrome, juvenile myelomonocytic leukemia, childhood myelodysplastic syndrome and myeloproliferative disorder, B-cell acute lymphoblastic leukemia, acute myelogenous leukemia, and in some types of solid tumors [19-21,55]. These genetic observations identify *PTPN11* as the first oncogene in the PTP superfamily. The majority of the gain-of-function mutations in SHP2 disrupt the autoinhibitory interactions between the N-SH2 domain and the PTP domain, which results in constitutive activation of SHP2 in the absence of a stimulus. The requirement of the SHP2 phosphatase activity for Ras activation provides a clear biochemical mechanism accounting for the malignant transformation observed in cells bearing gain-of-function SHP2 mutations. In addition, SHP2 has also been implicated in gastric carcinoma caused by the oncogenic bacterium *Helicobacter pylori*, which harbors a key virulence factor CagA that can promote SHP2 activation when it is tyrosine phosphorylated by the host Src family kinases [56]. Thus, SHP2 inhibitors are expected to have therapeutic values for a number of leukemias and solid tumors. Given the requirement of SHP2 function in multiple oncogenic receptor tyrosine kinase pathways, small molecule inhibitors may prove particularly effective for solid tumors with coactivation of receptor kinases, which respond poorly to kinase inhibitor monotherapy [57].

Although SHP2 represents an exciting target in cancer, few SHP2 inhibitors have been reported. Screening of the National Cancer Institute Diversity Set chemical library led to the identification of 8-hydroxy-7-(6-sulfonaphthalen-2-yl)diazonyl-quinoline-5-sulfonic acid (NSC-87877) (**VIII** in Figure 2) as a potent SHP2 inhibitor with an IC_{50} value of 0.32 μ M [58]. Compound **VIII** exhibited 5-475 fold selectivity over PTP1B, HePTP, DEPI, CD45, and LAR. Unfortunately, compound **VIII** also inhibited SHP1, a negative regulator of cytokine signaling, with similar potency. Nonetheless, it was shown that compound **VIII** blocked EGF-induced activation of SHP2, Ras, and Erk1/2 in cell cultures but did not block EGF-induced Gab1 tyrosine phosphorylation or Gab1-SHP2 association. It is anticipated that further effort

will yield more potent and selective SHP2 inhibitors with improved bioavailability for therapeutic applications.

4 Cdc25 Phosphatases

The Cdc25 phosphatases remove the inhibitory phosphates from Thr14 and Tyr15 of CDKs thereby function as positive regulators of cell cycle progression and play a central role in the checkpoint response to DNA damage [59-61]. Humans have three isoforms of Cdc25 termed Cdc25A, Cdc25B, and Cdc25C, and each plays a distinct role in the cell cycle. Given the critical importance of the Cdc25 phosphatases in cell cycle regulation, they have been linked to oncogenic transformation and human cancers in a variety of ways. For example, Cdc25B expression results in transformation of mouse embryonic fibroblasts and transgenic mice expressing Cdc25B in the mammary gland exhibit increased susceptibility to carcinogen-induced tumors [62,63]. Furthermore, both Cdc25A and Cdc25B have been implicated in the oncogenesis breast cancer, pancreatic ductal adenocarcinoma, prostate cancer, and non-Hodgkin's lymphoma [64-66], and elevated expression of Cdc25A and Cdc25B is thought to promote the loss of cell cycle check-point control, uncontrolled cell proliferation, and genetic instability [66]. Thus, Cdc25A and Cdc25B constitute attractive targets for cancer and there is considerable interest in the discovery of Cdc25 phosphatase inhibitors for cancer treatment [67].

Some of the initially described Cdc25 inhibitors consist of natural product derivatives. Dysidiolide, a natural product isolated from the marine sponge *Dysidea etheria* (**IX** in Figure 3), was identified as an inhibitor of Cdc25A with an IC₅₀ value of 9.4 μ M [68]. Dysidiolide also inhibited growth of A-459 lung carcinoma and P388 murine leukemia cells at micromolar concentrations, although the mechanism of growth inhibition by dysidiolide remains undefined. Further structure and activity analysis furnished a simplified analogue of the natural product (**X** in Figure 3), which showed both Cdc25A inhibition and growth inhibitory activity comparable to the parent compound [69]. Other examples of natural product derived Cdc25 inhibitors include the benzoquinoid dnacin B1 and coscinosulfate (**XI** and **XII** in Figure 3) [70,71]. It is fair to point out that these natural products display only modest potency (~10 μ M) with very limited selectivity toward the cdc25 phosphatases. In most cases/, the manners by which these compounds interact with Cdc25 are unclear, rendering structure-based optimization of new analogues difficult.

By far the most studied Cdc25 inhibitors are vitamin K3-like quinone derivatives. Vitamin K3 (menadione, **XIII** in Figure 3) was first noted to possess inhibitory activity against Cdc25 phosphatases [72]. Subsequent high-throughput screening campaigns led to the discovery of compound **XIV** (Figure 3), which displayed mixed inhibition kinetics with IC₅₀ values for Cdc25A, -B, and -C of 22, 125, and 57 nM, respectively [73]. Compound **XIV** showed significant growth inhibition against human and murine carcinoma cells and blocked G2/M phase transition. In addition to naphthoquinones, benzothiazolodiones (e.g. **XV** in Figure 3), quinolinediones (e.g. **XVI** in Figure 3), and indolyldihydroxyquinones (e.g. **XVII** in Figure 3) have also been shown to potent Cdc25 inhibitors [67,74]. In general, the quinone compounds afford potent Cdc25 inhibitors, some of which also exhibit growth inhibition in cell-based assays. The mechanisms of action often evoked for these compounds are either the irreversible oxidation of the cysteine present in the active site or the nucleophilic attack of electrophilic entities by the cysteine or one of the vicinal cysteines, leading to a covalent modification and inactivation of the enzyme [74]. Interestingly, although structurally similar to other quinone-based inhibitors, compound **XVII** inhibited Cdc25B reversibly and competitively with a submicromolar K_i value [75]. It was suggested that the rather electron-rich nature of the quinone moiety might disfavor the Michael addition reaction. However, despite the promising cell-based efficacies exhibited by the quinone-based Cdc25 inhibitors, there is concern about

toxicity induced by reactive oxygen species formed during the redox cycling of many quinone drugs. Identification of non-quinone inhibitors will be an important focus in future effort targeting the Cdc25 cell cycle regulators for new pharmacologic approaches in cancer therapy.

5 PRL Phosphatases

The PRL (phosphatase of regenerating liver) phosphatases represent a novel subfamily of PTPs, which are implicated in a number of tumorigenesis and metastasis processes [22]. PRL1 expression is elevated in several tumor cell lines, and cells expressing high levels of PRL1 exhibit enhanced proliferation and anchorage-independent growth [76,77]. Up-regulation of the related PRL2 and PRL3 also promotes cell growth and proliferation [78-81]. In addition to a role in cell proliferation, the PRLs are also involved in tumor metastasis. For example, the PRL3 message is amplified in colorectal cancer metastases, whereas its expression in primary tumors and normal colorectal epithelium is undetectable [82]. Subsequently, PRL3 mRNA level was found to be elevated in nearly all metastatic lesions derived from colorectal cancers, regardless of the sites of metastasis (liver, lung, brain, or ovary) [83,84]. High PRL3 expression has also been reported in cancer types other than colorectal cancers [22]. Furthermore, cells stably expressing PRL1 or PRL3 display enhanced motility and invasiveness, and induce metastatic tumor formation in mice [80,85]. Finally, alteration of PRL1 expression in a number of cancer cell lines leads to changes in cell adherence and invasive properties [86,87] and knockdown of endogenous PRL3 in cancerous cells using small interfering RNA can abrogate cell motility and the ability to metastasize in a mouse model [84,88]. Collectively, these studies strongly suggest that an excess of PRL3 is a key alteration contributing to the acquisition of metastatic properties of the tumor cells. As a consequence, PRL3 makes a very tractable target for small-molecule inhibitors designed to prevent and/or treat metastases.

Potent and selective PRL inhibitors may ultimately constitute a novel and effective family of anti-cancer agents. Unfortunately, very limited information for PRL inhibitor design is available in the literature. One report showed that the anti-leishmaniasis drug pentamidine (1,5-di(4-aminophenoxy)pentane) (**XVIII** in Figure 4) inhibited all three recombinant PRLs *in vitro* and caused tumor shrinkage in a melanoma mouse xenograft model [89]. Because pentamidine inhibited the activity of all three PRLs as well as other PTPs like PTP1B, SHP2, and MKP1, it is not clear if the inhibition of tumor growth was caused by the inhibition of a specific PRL, a combination of the PRLs, or another phosphatases. Moreover, it should be noted that pentamidine is a known DNA minor groove binder and has also been shown to disrupt hERG protein processing and hence lower functional hERG protein levels [22]. More recently, a number of rhodanine derivatives (e.g. **XIX** in Figure 4) [90] and biflavonoids (e.g. **XX** in Figure 4) isolated from the MeOH extract of the young branches of *Taxus cuspidata* [91] have been shown to inhibit PRL3 with IC₅₀ values in the low μ M range. Further studies are required to establish the selectivity profiles and the modes of action (i.e., competitive/noncompetitive and reversibility) for the compounds.

In addition to targeting the PTP active site for inhibitor development, recent structural and biochemical studies suggested an alternative approach to block the PRL-mediated processes. One of the most striking features of PRL1 in comparison with other PTPs is that it exists as a trimer in the crystalline state [92,93]. It appears that trimerization may be a general property for all PRL enzymes, and that PRL1 trimer formation is essential for PRL1-mediated cell growth and migration [94]. The functional requirement for PRL trimerization suggests a novel mechanism for PTP regulation. In addition, the trimeric interface presents a unique opportunity for the development of small molecule compounds designed to disrupt PRL trimerization. This offers a distinct advantage comparing to the traditional approach to target the active sites, which are highly conserved among the PTPs. Thus, targeting the PRL trimerization interface may

present an alternative strategy for developing selective PRL inhibitors with improved pharmacological properties.

6 Concluding Remarks

Abnormal cellular signaling associated with aberrant tyrosine phosphorylation is a hallmark of cancer. Not surprisingly, abnormal expression and/or activation of PTKs have been associated with a number of oncogenic processes. Several PTK inhibitors have reached the clinic validating the notion that cellular pathways regulated by tyrosine phosphorylation could be exploited for cancer treatment. The PTPs play critical role in regulating the level of protein tyrosine phosphorylation and have distinct (and often unique) biological functions *in vivo*. In addition, as observed with PTKs, deregulation of PTP activity also contributes to the pathogenesis of cancer. Cancer-related PTP perturbations range from loss of expression to point mutation and single amino acid substitution. There are also examples of amplification, overexpression and ectopic expression of PTPs in cancer. Considerable evidence has now accumulated that suggests that PTP1B, SHP2, Cdc25 and PRL phosphatases represent highly attractive targets for cancer therapy. Inhibitors of the PTPs are expected to have therapeutic value with unique modes of action.

With many PTPs identified as cancer targets, a pressing challenge for PTP-based therapeutics is to obtain highly potent and selective small molecule inhibitors with excellent bioavailability. Interests in developing PTP inhibitors have increased considerably over the last few years. A number of strategies are currently being explored for the identification of various classes of PTP inhibitors. These efforts have resulted many potent, and in some cases selective, inhibitors for PTP1B, SHP2, Cdc25 and PRL phosphatases. Structural information derived from these compounds serves as a solid foundation upon which novel anti-cancer agents targeted to these PTPs can be developed. In addition, potent and selective inhibitors targeted to this group of PTPs will serve as powerful tools to delineate the function of these phosphatases in normal physiology and in pathological conditions. Obtaining this knowledge is vital for understanding the PTP-mediated tumor growth and metastasis, and for the development of novel anti-cancer therapies targeted to these enzymes.

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Abbreviations

PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
SH2	Src homology-2
PRL	phosphatase of regenerating liver

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Figure 1.
Representative PTP1B inhibitors.

VIII

Figure 2.
A SHP2 inhibitor.

Figure 3.
Representative Cdc25 inhibitors.

Figure 4.
PRL inhibitors.