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Targeting Protein Tyrosine Phosphatases for Anticancer Drug Discovery

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Abstract

Protein tyrosine phosphatases (PTPs) are a diverse family of enzymes encoded by 107 genes in the human genome. Together with protein tyrosine kinases (PTKs), PTPs regulate various cellular activities essential for the initiation and maintenance of malignant phenotypes. While PTK inhibitors are now used routinely for cancer treatment, the PTP inhibitor development field is still in the discovery phase. In this article, the suitability of targeting PTPs for novel anticancer drug discovery is discussed. Examples are presented for PTPs that have been targeted for anticancer drug discovery as well as potential new PTP targets for novel anticancer drug discovery.

Keywords

Protein tyrosine phosphatase inhibitor; Shp2; PTP1B; Cdc14; PRL-3; LMW-PTP; Cdc25; Eya

INTRODUCTION

Discovered three decades ago, protein tyrosine phosphorylation is now well recognized as a major regulatory mechanism of cell signaling and activities [1]. Aberrant tyrosine phosphorylation is either the primary cause of human cancer or is required for maintaining the oncogenic state that include abnormal survival, replication, growth, metastasis, angiogenesis, resistance to growth inhibition, evasion of immune surveillance, and stress support of human cancer [2,3]. Protein tyrosine phosphorylation is controlled reciprocally by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Many PTKs have been identified as human oncogenes and are validated targets for cancer therapy. Small molecule PTK inhibitors such as Bcr-Abl inhibitors imatinib and dasatinib, epidermal growth factor receptor (EGFR) inhibitors gefitinib and erlotinib are in clinical use for human cancer treatment. Whereas the PTK-targeted therapy field has advanced to the United States Food and Drug Administration (FDA)-approved drugs, PTP inhibitor development is in the early discovery phase. In this article, we discuss evidence that specific PTPs are targets for development of novel anticancer drug and summarize selected PTP drug discovery efforts. Readers are referred to several previous review articles of PTPs and PTP inhibitors for additional information [4-9]. Table I lists PTP inhibitors that have been tested *in vivo*. For comprehensive description of PTP classification, the articles by Alonso and colleagues [10] and Andersen and colleagues [11] are recommended.

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PROTEIN TYROSINE PHOSPHATASES AS ANTICANCER DRUG TARGETS

At least three criteria need to be considered when selecting a PTP as the direct target for novel anticancer drug discovery:

- **A.** Does the PTP play a positive role in the pathogenesis of human cancer such that inhibition of its activity suppresses malignant phenotypes? This is fulfilled if the PTP is an oncogene that causes human cancer or is a non-oncogene addiction gene required for maintenance of malignant phenotypes.
- **B.** Alternatively, does the PTP contribute to resistance to an anticancer therapy used in the standard care of cancer patients such that inhibition of its activity sensitizes the anticancer therapy?
- **C.** Does the potential PTP drug target have appropriate surface properties (surface topology, charge distribution, lipophilic potential) at its active site and the surrounding area such that high affinity and specific binding of a small molecule inhibitor may be achieved?

Although it is not impossible, small molecule activators of proteins are much more difficult to obtain than small molecule inhibitors. Drug discovery efforts generally aim at development of inhibitors. Since aberrant activation of many PTKs is associated with various types of human cancer and PTPs catalyze the reverse reaction of PTKs, PTPs were initially thought as negative regulators of cancer phenotypes [4,12,13]. Thus, inhibition of PTPs would be predicted to facilitate, rather than suppress, oncogenesis. This concept has proven to be inaccurate. Biological systems are much more complex than the simple *in vitro* chemical reaction (Fig. (**1**)). While many specific tyrosine phosphorylation sites on proteins serve as positive signals to propagate activating responses, some of these tyrosine phosphorylation sites also trigger negative-feedback mechanism to terminate the activation signal. Moreover, certain tyrosine phosphorylation sites have suppressive effect on enzyme activities. For example, phosphorylation of human c-Src at Tyr-530 by Csk tyrosine kinase inhibits the c-Src tyrosine kinase activity. Dual phosphorylation of Cdk1 at Thr-14 and Tyr-15 blocks its kinase activity. Dephosphorylation of these residues leads to enzyme activation. In fact, increasing evidence suggests that cell signaling requires coordinate action of both PTK and PTP activities [5]. Therefore, PTPs could cooperate with PTKs, in addition to antagonizing them, in promoting cancer growth and progression.

Another dogma contributing to the slow start of PTP drug discovery efforts was that PTKs are highly regulated and specific, whereas a few constitutive, non-specific PTPs passively counteract the function of PTKs [14]. It is now known that there are at least 107 PTP genes in the human genome, providing highly regulated and specific function in various types of human cells [10,13]. Human PTPs are grouped into three classes of Cys-based PTPs and a fourth family of Asp-based PTPs. Although designated as PTPs, besides phosphotyrosine-specific phosphatases, PTPs include dual specificity phosphatases (DSPs) that dephosphorylate protein tyrosine and serine/threonine residues and phosphatases that their known physiological substrates are phosphothreonine residues, phospholipids, and mRNA. Among Class I phosphotyrosine-specific classical PTPs, the transmembrane PTPα (encoded by the *PTPRA* gene) is an activator of c-Src. The non-receptor PTP Shp2 (*PTPN11*) is a positive regulator of growth factor signaling. Gain-of-function Shp2 mutants have been established as oncogenes. Both positive and negative effects of PTP1B on tumorigenesis have been reported. Cell cycle requires at least three groups of PTPs to modulate Cdks and their substrates: Class III PTPs Cdc25s and Class I dual-specific PTPs Cdc14s, and Kap (*CDKN3*). Cdc25s dephosphorylate the dual Thr-Tyr phosphorylation sites at the N-terminal region of Cdks to activate these kinases to drive the cell cycle progression. Cdc14s regulate mitosis exit and centrosome separation. Cdc25 and Cdc14, therefore, are potential targets for

inhibition of cell proliferation. Kap dephosphorylates the activating Thr-160 of Cdk2 and thus is a Cdk inactivator [15]. While the role of Kap in tumorigenesis is controversial [16,17], CDKN3 mRNA is frequently elevated in human cancer. Furthermore, inactivation of Cdk2 is required for mitotic exit in some organisms [18]. The Class I dual specific PTP PRL-3 (*PTP4A3*) promotes cancer metastasis [19]. The low molecular weight PTP (LMW-PTP, *ACP1*) is the sole member of the Class II PTP. Overexpression of LMW-PTP is sufficient to transform MCF-10A mammary epithelial cells [20] and NIH3T3 fibroblast cells [21]. Recent studies have shown that phosphorylation of Tyr-142 at the C-terminal region of γ-H2A.X histone prevents DNA-damage repair and induces apoptosis [22,23]. The Asp-based PTPs Eya1 and Eya3 are responsible for dephosphorylation of Tyr-142 on γ-H2A.X [23,24]. Conceivably, blocking Eya1/Eya3 PTP activity could be used to enhance the therapeutic efficacy of DNA damage-based cancer therapy. Thus, candidates of anticancer drug targets are found in every family of PTPs.

In a study employing RNA interference screen to identify anti-apoptosis genes in Hela cells, 28 of the 107 human PTPs genes were found to be positive regulators of cell survival, whereas only 4 PTPs were identified as cell death phosphatases [25]. Thus, the number of anti-apoptotic PTPs is 7-times of the number of pro-apoptotic PTPs in Hela cells. The overall number of PTP genes contributing to the survival of human cancer cells is likely to be higher because Hela cells are likely to express only a portion of the human phosphatome and because different requirements for survival in various types of cancer cells of diverse genetic origin. Therefore, there are several known and likely many yet to be identified PTPs that satisfy Criteria **A** and **B** described above.

Needless to say, some of PTPs are established or potential tumor suppressors [4,13]. Mutational and promoter methylation analyses have provided links between several PTP genes to various types of human cancer. These include PTEN, PTPRF, PTPRG, PTPRJ, PTPRO, PTPRT, PTPN3, PTPN6, PTPN13, PTPN14, and DUSP6 [5,13]. Among these PTPs, Pten is clearly established as a tumor suppressor in various types of human cancer through extensive investigation that includes mouse models of tumorigenesis [4,5]. Notably, physiological substrates of Pten are phosphoinositide 3-phosphates, not proteins. There is substantial evidence that DEP1 (PTPRJ) is a tumor suppressor [4,13]. Roles of other PTPs as tumor suppressors are less well-established. If some of them are confirmed as tumor suppressors and if the loss-of-function is due to epigenetic silencing, indirect re-activation through epigenetic approaches such as the use of demethylating agents can be explored to restore expression of these PTP tumor suppressors as an anticancer strategy.

The potential tumor suppressor function of some PTPs raises the concern about potential cross-inhibition of these PTP tumor suppressors by poorly selective PTP inhibitors. This concern can be addressed by answering three questions. First, does the PTP target have distinct surface properties surrounding the active site to allow selective binding of small molecule inhibitors? Second, if cross-inhibition of a small molecule PTP inhibitor cannot be completely avoided, does inhibition of the targeting PTP have a dominant effect over inhibition of the cross-inhibited PTP? Third, is cross-inhibition of a putative PTP tumor suppressor in normal cells sufficient to turn normal cells cancerous? It should be noted that, since tumor suppressors are defective or absent in cancer cells, a drug that cross-inhibits a tumor suppressor in cancer cells under this condition has no functional significance.

The first question brings up Criteria **C** described above. Crystal structures of catalytic domains of Class I Cys-based PTPs share a similar Cα-backbone trace [11,26]. Despite the overall conserved ternary structure, comparison of the surface surrounding these PTP catalytic sites revealed diverse topology, electrostatic potential, and lipophilic potential characteristics [10,26,27]. Barr et al. mapped conserved residues of all experimental

structures of PTPs onto the surface of PTP1B and the D1 domain of RPTPμ [26]. They found only a few conserved surface patches surrounding the active site. These distinct surface properties exist even between close members of PTPs, such as Shp1 and Shp2 [26,27]. Thus, PTPs have different properties on the surface surrounding the catalytic site that allow development of specific inhibitors. In support of this notion, selective PTP inhibitors have been reported [28,29].

A challenge in targeting the PTP catalytic site is the relatively shallow pocket of the catalytic site. Nevertheless, after the discovery that PTP1B has a secondary substrate binding pocket [30,31], high affinity bidentate inhibitors with Ki < 10 nM have been developed [32] (see Fig. (**2**)). In a recent large-scale structure analysis of catalytic domains of classical PTPs, it was revealed that many PTPs have a secondary pocket proximal to the catalytic site [26]. Therefore, the limitation of the pocket depth of the catalytic site of PTPs can be overcome by utilizing bidentate inhibitors that bind simultaneously to the catalytic and the secondary pockets.

Selective PTP inhibitors that display >10-fold selectivity against similar PTPs and >100-fold against other PTPs are obtainable. However, a monospecific PTP with >100-fold selectivity against all other PTPs has yet to be identified. Therefore, an issue needed to be considered in the PTP anticancer drug discovery is whether inhibition of the targeting PTP has a dominant effect over inhibition of the cross-inhibited PTP, particularly if the cross-inhibited PTP is a putative tumor suppressor. In general, a PTP is considered as a target for anticancer drug discovery because it is required for survival, proliferation, or metastasis of cancer cells or it contributes to drug resistance. Inhibition of the single PTP target alone in cancer cells by molecular biology approaches is sufficient to suppress malignant phenotypes. Thus, inhibition of the targeting PTP is predicted to have a dominant effect on suppression of cancer cells. Although further study is required to address this issue, we are not aware of any evidence that any PTP inhibitor can promote cancer cell survival and growth.

Similarly, the synthetic effects of inactivation of a tumor-promoting PTP and a tumor-suppressor PTP in normal cells remain to be studied. Asides from genetic/epigenetic links, the role of a PTP as a tumor suppressor is generally assessed by the expression of a functional PTP in cancer cells. There is paucity of evidence that inactivation of a putative PTP tumor suppressor is sufficient to cause cancer, except the phosphoinositide phosphatase Pten. For instance, *PTPRJ* (DEP1 gene)-null mice do not develop spontaneous tumor [33]. Therefore, although pre-clinical and clinical evaluations will be required, it is predicted that a selective PTP inhibitor, even if it weakly cross-inhibits a putative PTP tumor suppressor, is unlikely to cause therapy-induced tumor and therefore it is acceptable as an anticancer drug candidate in this regard.

Another issue is the potential toxicity of inhibiting the targeted PTPs in normal cells. Although this needs to be tested in each case through clinical trials, it is believed that therapeutic windows exist for exploration of selective toxicity to cancer cells. PTPs selected as drug targets usually are aberrantly active in the cancer cells, which may confer specific dependency of cancer cells to the PTPs. For instance, it has been reported that Shp2 knockdown specifically inhibits primary chronic myeloid leukemia (CML) cells but not normal $CD34^+$ cells [34]. Furthermore, for certain terminal diseases, short term, low grade toxicity with drugs that have proven benefits to the disease management may be acceptable.

In the following sections, we describe Shp2 as a target for novel anticancer drug discovery and summarize other established and potential PTP targets for anticancer drug discovery.

SHP2 (*PTPN11*) AS AN ANTICANCER DRUG TARGET

Shp2 is a Class I non-receptor classical Cys-based PTP containing tandem Src homology-2 (SH2) domains [35]. The presence of phosphotyrosine-binding SH2 domains provides an immediately recognizable link to PTK signaling when it was cloned by several laboratories in 1992-1993. Shp2 is a homolog of *Drosophila* corkscrew (csw) gene product. Shortly after mammalian Shp2 was cloned, several laboratories tested effects of catalytic-Cys mutated Shp2 on insulin- or epidermal growth factor (EGF)-stimulated Ras and Erk1/Erk2 (Erk1/2) activation. These experiments consistently showed that a catalytic-inactive Shp2 displayed a dominant-negative effect on insulin- or EGF-induced Ras/Erk1/2 activation [36,37]. These and other experiments documented Shp2 as a positive mediator of mitogenic signaling of growth factor receptor tyrosine kinases and showed that the PTP activity is essential.

Structure

Human Shp2 contains two SH2 domains in the N-terminal region, a single PTP domain following the tandem SH2 domains, and a C-terminal region with Tyr phosphorylation sites. Two Shp2 [593 amino acid (aa) and 597 amino acid residues] exist in humans and rodents that differ in a stretch of 4 amino acid residues in the catalytic domain. These two Shp2 are derived from a single gene and are believed to be functionally identical. The tandem SH2 domains are designated as N-SH2 (aa 2-104) and C-SH2 (aa 112-215) domain, respectively. Shp2 SH2 domains preferred a consensus binding motif of (T/V/I/y)-X-pY-(A/s/t/v)-X-(I/V/L) [38,39]. Cys-459 in the catalytic domain (aa 220-525) is the catalytic Cys. The mechanism of tyrosine dephosphorylation is illustrated in Fig. (**3**). The C-terminal region has two tyrosine residues (Tyr-546 and Tyr-584) that may be phosphorylated after growth factor stimulation, especially PDGF and FGF. These two tyrosine residues are located within Grb SH2 domain binding motif (pYxNx) and are reported to mediate Grb2 binding.

The crystal structure [40] of human Shp2 apo-protein (aa 1-525, without the C-terminal region) shows that the phosphotyrosine binding sites of SH2 domains face outward away from the catalytic domain while the backside of N-SH2 domain (primarily via the D'E loop) wedges into the catalytic pocket (Fig. (**4**)). Thus, unliganded Shp2 is auto-inhibited by its N-SH2 domain and has a low basal PTP activity. Binding of Shp2 SH2 domains to specific phosphotyrosine containing peptides induces a conformational change that relieves the intramolecular autoinhibition. This is predicted from crystal structures [40,41] and based on experimental evidence [42]. The tandem SH2 domains are necessary for high affinity binding to bisphosphoryl tyrosine-based activation motifs (BTAMs) [42-44].

Binding of Shp2 to specific BTAMs such as those located on Gab family of docking proteins are required for its biological function. Deletion of N-SH2 domain or both SH2 domains results in fully activated Shp2. However, these deletion mutants are not biologically functional. Similarly, point-mutations that disrupt the N-SH2 domain or C-SH2 domain binding activity render the mutant Shp2 biologically non-functional. In contrast to the catalytic Cys-mutant, these SH2 domain-defective Shp2 mutants do not have a dominant effect over endogenous wildtype Shp2.

Besides the crystal structure of full length Shp2 (lacking the C-terminal region) [40], a crystal structure of the Shp2 PTP domain in complex with malic acid was published recently [26]. In these crystal structures, the catalytic WPD loop is in an inactive "open" conformation [26] (Fig. (**5**)). In contrast, the WPD loop of the closely related Shp1 PTP is in an active "closed" conformation in the absence of a substrate in crystal structures [26,27]. The open conformation of the Shp2 WPD loop in crystals may be due to a lower energy state of this conformation in the absence of substrate or because of spatial constraint in these crystals. Enzyme kinetic studies have shown that the Shp1 catalytic domain is a much more

active enzyme than the Shp2 catalytic domain *in vitro* [45,46], suggesting that the Shp2 catalytic domain is in a less optimal, perhaps "open" conformation in aqueous solution similar to that observed in the crystal structures. The distinct WPD loop conformation of these two PTPs contributes to the different surface charge distribution at areas surrounding the catalytic site [26]. This may provide a ground for PTP inhibitor selectivity. Similar to PTP1B, the Shp2 PTP domain has a secondary substrate binding pocket proximal to the catalytic site [26]. Thus, it may be possible to obtain highly potent, bidentate Shp2 PTP inhibitors that dock into these two sites simultaneously in analogy to PTP1B inhibitors [47].

Pathways and substrates

The most consistently reported Shp2-regulated signaling pathway is the Ras-Erk1/2 mitogen-activated (MAP) kinase pathway. Increased peak signal strength, prolonged activation period, or both have been observed. This includes Ras-Erk1/2 activation by various growth factor receptor tyrosine kinases such as EGFR, ErbB2, c-Met, PDGFR, insulin, FGFR1, cytokine receptors such as GM-CSF and IL-6, G-protein-coupled receptors such as lysophosphatidic acid, tyrosine kinase oncogenes such as Bcr-Abl, and oncogenic bacterial protein CagA. Shp2 positively regulates the Ras-Erk1/2 MAP kinase pathway is supported by evidence obtained using dominant-negative Shp2 mutants, Shp2 knockdown with RNA interference, Shp2-defective mouse embryo fibroblast cells, Shp2 knockout mice, gain-of-function Shp2 mutants in cell cultures and in animals. Shp2-mediated Ras-Erk1/2 MAP kinase pathway is involved in regulation of cell survival, proliferation, differentiation, adhesion, and migration depending on cell contexts.

Shp2 binds directly to PDGFRβ. Only one Shp2 binding site on PDGFRβ cytoplasmic region is known. It is believed that Shp2 binds to a PDGFR dimer to achieve sufficient affinity but this has not been proven. In most growth factor- and cytokine-simulated cells, including PDGF-stimulated cells, Shp2 binds to PH-domain containing docking proteins Gab1 and/or Gab2 through interaction between the Shp2 SH2 domains and a BTAM located at the C-terminal region of Gab proteins [42]. Disruption of Gab1 and Shp2 binding impairs EGF-stimulated Erk1/2 activation [42]. Conversely, expression of a Gab1-Shp2 fusion protein results in constitutive Erk1/2 activation [42,48]. In addition to Gab family of proteins, Shp2 binds to fibroblast growth receptor substrate 2α (FRS2α) in FGF-stimulated cells and to insulin receptor substrate-1 (IRS-1) in insulin-stimulated cells [49-51]. Shp2-FRS2α interaction is involved in FGF-stimulated Erk1/2 activation while the role of Shp2-IRS-1 interaction in Erk1/2 activation is less clear.

How Shp2 activates the Ras-Erk1/2 pathway is not completely resolved although both receptor tyrosine kinase specific and shared mechanisms have been reported (Fig. (**6**)). It has been found that Shp2 activates Src family kinases (SFKs) by dephosphorylating Csk docking proteins paxillin [52] or Cbp/PAG [53]. Dissociation of Csk from Src leads to Src activation. However, the link from an activated Src to Ras activation remains a subject of investigation. Shp2 is recruited to paxillin by Gab1. It is unclear how Shp2 is recruited to Cbp/PAG.

It was reported that Shp2 dephosphorylates p120RasGAP binding sites on EGFR [54] or ErbB2 [55] to prevent membrane localization of p120RasGAP via these ErbB proteins. This is analogous to corkscrew binding to Torso in the *Drophophila* PDGFR pathway [56] and is receptor-specific. It was also reported that p120RasGAP binds to tyrosine-phosphorylated Gab1 and Shp2 prevents such binding by dephosphorylation the tyrosine phosphorylation site thereby activate Ras [57]. Shp2 was reported to dephosphorylate Sprouty in FGF-stimulated cells that results in dissociation of Grb2 from Sprouty [58].

Several transmembrane glycoproteins bind Shp2. These include Shp substrate 1 (SHPS-1)/ Signal regulatory protein (SIRP) [59], PECAM-1/CD31 [60], myelin protein zero-like-1 (MPZL1/PZR) [61] and several immunoreceptors that contain immunoreceptor tyrosine-based inhibitor motif (ITIM) such as FcγRIIB [62]. In contrast to specific binding of non-transmembrane docking proteins to Shp2, these transmembrane proteins bind both Shp1 and Shp2 and, in some cases, the phosphoinositide 5-phosphatase SHIP.

Shp2 is a regulator of cell adhesion and migration. These functions are mediated by dephosphorylation of focal adhesion kinase (FAK), activation of Src kinase, and the Ras-Erk1/2 pathway in addition to transcription-mediated events. Shp2 was reported to bind β4 integrin in Met-activated cells, which also activates c-Src [63].

While Shp2 is a positive regulator of cell growth and migration, it is a negative regulator of interferon signaling. Interferons induce STAT1 or STAT1/STAT2 tyrosine phosphorylation. STAT1 is a Shp2 PTP substrate [64]. Increased IFN-γ-induced responses are observed in mouse embryo fibroblasts lacking Shp2 [64,65].

Roles in cancer

Shp2-regulated Ras-Erk1/2 MAP kinase pathway, SFKs, and FAK are involved in tumor growth and metastasis. Overexpression of Shp2 has been observed in infiltrating breast carcinoma [66] and in primary leukemia cells [67]. It was reported that inhibition of Shp2 with a catalytic-inactive Shp2 mutant (Shp2C459S) abolished anchorage-independent growth of breast cancer cell lines and knockdown of Shp2 in BT20 and BT474 breast cancer cells resulted in a normal breast epithelial morphology [68]. Activating mutations of FGFR3 are associated with bladder carcinoma and multiple myeloma. It was reported that transformation of NIH3T3 cells by a constitutively active FGFR3 mutant is inhibited by a Shp2 C459S mutant [69]. The aberrant EGFRvIII (deletion exon 2-7) mutant is detected in 40-50% of human glioblastoma. EGFRvIII is also detected in about 5% of human lung squamous cell carcinoma [70]. Soft agar colony formation and tumor growth in nude mice of EGFRvIII-expressing glioblastoma cells are inhibited by a Shp2C459S mutant [71], indicating that Shp2 PTP activity contributes to EGFvIII-induced cell transformation and tumor growth. The Shp2 docking protein Gab2 is a Bcr-Abl substrate. In fact, it was originally cloned as a Shp2-associated tyrosine-phosphorylated protein from Bcr-Abl-transformed cells. It has been reported that proliferation of Bcr-Abl$^+$ CML cell lines and primary CML CD34+ cells, but not normal CD34+ cells, is inhibited by Shp2 shRNAs [34]. Anaplastic large cell lymphoma is associated with NPM-ALK fusion gene. Shp2 was found to bind NPM-ALK oncogene and regulated anaplastic lymphoma cell growth [72]. Virulence factor CagA-positive *H. pylori* are carcinogenic bacteria that cause gastric cancer. After injecting into human gastric epithelial cells, the membrane-associated CagA becomes tyrosine phosphorylated and recruits Shp2 to induce gastric epithelial cell transformation [73]. Together, these findings suggest that Shp2 is required for cell transformation and malignant growth of several oncogenic tyrosine kinases and the bacterial oncogenic protein CagA. The list of tyrosine kinase oncogenes known to be dependent on Shp2 for their malignant activities is likely to increase in the coming years.

While the wildtype Shp2 may function as a non-oncogene addiction gene, gain-of-function Shp2 mutants have been established as oncogenes. Mutations in the *PTPN11* gene that encodes Shp2 were originally discovered in the childhood development disorder Noonan syndrome as germline mutations [74]. Subsequently, somatic mutations in *PTPN11* genes were identified in 35% of juvenile myelomonocytic leukemia (JMML), 10% of childhood myelodysplasic syndrome (MDS), 7% of B-cell acute lymphoblastic leukemia (B-ALL), 4% of childhood acute myelogenous leukemia (AML), and in some cases of colon cancer, lung cancer, melanoma, and neuroblastoma [75-77]. JMML is an aggressive myeloproliferative

disorder (MPD)/MDS. JMML cells are hypersensitive to GM-CSF. Ras and neurofibromin (*NF1*) mutations together are associated with 50% of JMML patients. Thus, *PTPN11* is the most frequently mutated genes in JMML patients. Cancer-associated Shp2 mutations occur most frequently in N-SH2 domain in residues that affect N-SH2 domain and PTP domain interaction. Cancer-associated Shp2 mutations either have been demonstrated or are predicted to be gain-of-function mutants. No loss-of-function Shp2 mutant has been found in human cancer. Several laboratories have reported leukemia-associated Shp2 mutants (E76K, D61Y, D61V) are able to confer GM-CSF and IL-3 hypersensitivity of murine hematopoietic progenitor cells from fetal liver or bone marrow and evoked GM-CSF-independent colonies [78-80]. The Shp2 PTP activity and both SH2 domains are required for the transformation activity. In Balb/c mice, transplant of bone marrow cells infected with Shp2 mutant-encoding retroviruses results in fatal JMML-like disease and lymphoproliferation [80]. In human cytokine-dependent TF-1 myeloid cells, expression of the leukemia-associated Shp2 E76K mutant results in cytokine-independent transformation of these cells [81]. Recently, Chan et al reported that conditional expression of the leukemia-associated Shp2 D61Y mutant in hematopoietic cells of knockin mice led to a fatal MPD [82]. Thus, mutant Shp2 has been established as an oncogene in leukemia.

Small molecule Inhibitors

By screening the NCI Diversity Set-1 library, NSC-87877 (**1**, Fig. (**7**)) was identified as a Shp2 PTP inhibitor with an IC_{50} of 0.33 μM [83]. NSC-87877 inhibits Shp1 with the same potency. It is 5- and 24-fold less potent at inhibiting PTP1B and HePTP, respectively, and displays >200-fold selectivity against DEP1, CD45, and LAR. Molecular docking analysis suggests that NSC-87877 forms hydrogen bonds with the backbone NH group of Arg-465 in the PTP signature motif and with side-chains of Lys-280 and Asn-281. Mutational experiments with the non-conserved Lys and Asn suggested that these two residues are involved in the interaction with NSC-87877. Comparison of NSC-87877 sensitivity of wildtype Shp2, Shp2E76K mutant, and Shp2 PTP domain indicated that the wildtype Shp2 is ~4.5-fold less sensitive to NSC-87877. Because the catalytic pocket of the wildtype Shp2 is blocked by N-SH2 domain, the required higher concentration of NSC-87877 necessary to achieve the same degree of inhibition of wildtype Shp2 supports the notion that NSC-87877 binds to the catalytic pocket. NSC-87877 has been reported to inhibit Shp2 in epithelial/carcinoma cells, endothelial cells, fibroblasts, muscle cells, and neuronal/glioma cells [71,83-86]. However, NSC-87877 does not appear to be able to enter hematopoietic cells. With two arylsulfonic acid groups, we suspect that NSC-87877 is a substrate of the organic anion transporter (OATP) family of membrane transporters, although this has not been proven experimentally.

Based on another hit, NSC-117199 (**2a**), (Shp2 IC_{50} = 47 μM) from a Shp2 PTP inhibitor screen of the NCI Diversity Set-1 chemical library, >200 analogs were synthesized [29]. The focused library synthesis involved exploitation of sulfonic acid moiety and the nitro group in the NSC-117199 scaffold to generate potent compounds with IC_{50} values of around 1 μM (e.g. **2b**). The library of isatins contained polar replacements of the sulfonic acid of **2a** phosphotyrosine mimics. The docking pose of isatin **2b** is shown in Fig. (**8**). In this case the compounds docks best to the Shp2 catalytic site with the *ortho*-carboxylate bound close to the catalytic cystein residue. Similarly, the nitro group in NSC-117199 was replaced with more stable carboxylic acid moiety. These moieties appear to be essential for Shp2 inhibitory activity and provide a pharmacophore for further development of novel compounds for Shp2. Several compounds have IC_{50} to Shp2 ~ 1 μM, with various degrees of selectivity against Shp1 and PTP1B were identified. Two compounds from this series (**3** and **4**), have > 10-fold selectivity against Shp1 and PTP1B.

Using a homology model of Shp2 PTP domain based upon a crystal structure of a PTP1B in complex with a potent small molecule inhibitor, Birchmeier and colleagues performed virtual screen of ~2.7 million compounds and identified phenylhydrazonopyrazolone sulfonate 1 (**5**) (PHPS1) and PHPS4 (**6**) as Shp2 selective inhibitors [28]. PHPS1 is less potent (Shp2 IC_{50}: 2.1 μM) than PHPS4 (Shp2 IC_{50}: 0.63 μM) but has greater selectivity against other PTPs. The phenyl sulfonate group of PHPS1 appears to be essential for the inhibitor activity. The computer model suggests that the phenyl sulfonate group inserts into the active site and forms hydrogen bonds with ILE-463, Gly-464, Arg-465, Ser-460, and Ala-461, whereas the pyrazolone core and two other phenyl rings make contacts with residues at the periphery of the catalytic pocket (Fig. (**9**)). The model is supported by mutational studies using PTP1B and Shp2 mutants. PHPS1 is able to inhibit Shp2 PTP in several cell-based assays [28].

By screening a natural product-based compound collection, Waldmann and colleagues identified a furanodictine class of Shp2 inhibitor (**7**, Shp2 IC_{50}: 2.5 μM) that is highly selective against PTP1B (PTP1B IC_{50}: >100 μM) [87]. With >40-fold selectivity against PTP1B, compound (**7**) represents the most selective Shp2 inhibitor against PTP1B identified so far. Three other less selective furanodictine compounds were also identified that inhibited both Shp2 and PTP1B with IC_{50} <10 μM. It is unknown whether these are competitive Shp2 inhibitors or how they interact with the Shp2 PTP domain. No cell-based experiment was reported in that study so that it is unclear whether these compounds are cell permeable or are indeed cell active in a Shp2-dependent manner.

Geronikaki et al synthesized and tested three series of 2-thiazolylimino/ heteroarylimino-5arylidene-4-thiazolidinones with thiazole-, benzo[d]thiazole-, or benzo[d]isothizole rings as Shp2 inhibitors [88]. Computer docking suggests that the phenyl ring is oriented to the catalytic pocket. The most potent compounds (**8** and **9**, both have a Ki against full length GST-Shp2 of 11.7 μM as measured by the pNPP assay) have a 4-methoxyl-substitued on the phenyl ring. These compounds do not contain a negatively charged sulfonic acid or carboxylic acid group featured in many PTP inhibitors. However, the pKa of the thiazolidinone may be sufficiently low in these systems to render these compounds ionic under physiological conditions. Nevertheless, these compounds are > 10-times less potent than NSC-87877, compound (**4**), and PHPS1, which have sub-micromolar Ki to Shp2 ([28] and our unpublished data). The selectivity and cell permeability of these compounds were not reported.

Based on a putative binding pocket of Shp2 for substrate pY+5 residues, which is different between Shp1 and Shp2, Yu et al screened a database of 1.3 million compounds by docking into this secondary pocket [89]. Compound (**10**) (Fig. (**10**)) was identified that inhibits Shp2 in a PTP assay using an insulin receptor-derived decapeptide as the substrate (IC_{50}: ~100 μM) but did not inhibit Shp1 at 100 μM. If the binding mode is confirmed, this may provide a precursor for synthesizing bidentate inhibitors in combination with inhibitors that bind to the catalytic site.

Inhibitors designed for PTP1B that cross-inhibit Shp2 have also been reported. These include a bidentate PTP1B inhibitor (**11**), (PTP1B *Ki*: 2.4 nM; Shp2 IC_{50}: 10 μM) [47] and a bis(trifluoromethanesulfonamide) (**12**) (Shp2 IC_{50}: 1.8 μM). Gobert et al synthesized two Glepp1 PTP inhibitors (**13** and **14**, IC_{50}: 0.12 μM, 0.34 μM) that potently cross-inhibit Shp2 (IC_{50}: 0.39 μM, 0.65 μM) [90]. Compounds (**13**) and (**14**) are orally bioavailable and have protective effect on dextran sulfate sodium-induced ulcerative colitis in mice [90]. In an effort to develop retinoid-derived orphan nuclear receptor small heterodimer partners that induce apoptosis in leukemic cells, compound (**15**) was found to inhibit Shp2 (IC_{50}: 0.45 μM) [91].

Assessment of target inhibition

PTP activity assay in vitro—PTP inhibitors are usually screened by *in vitro* PTP activity inhibition assay [14]. Shp2 PTP activity may be assayed *in vitro* using the fluorogenic 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) (available from Invitrogen). DiFMUP is non-fluorescent and is hydrolyzed to the fluorescent DiFMU (maximal excitation/emission at ~358/450 nM) by the PTP. The fluorescent signal can be read without stopping the PTP reaction. In chemical library screening, potential hits may be missed if these compounds are fluorogenic. The chromogenic *p*-nitrophenyl phosphate (pNPP) is another generic PTP substrate frequently used in PTP activity assay *in vitro*. Hydrolysis of pNPP yields the yellow colored *p*-nitrophenol at alkaline pH that can be measured with a spectrophotometer at a wavelength of 405 nm. A potential problem is that some of screening samples may be colored and may interfere with the assay readout. Thus, appropriate controls without the PTP should be included in a confirmation screen. Alternatively, hydrolysis of pNPP can be determined by measuring the liberated free phosphate with the Malachite green assay [42] or the use of Biomol Green reagent (available from Biomol). Free-phosphate contamination is the major interference using the Biomol Green reagent. The pNPP-based assays require stopping of reaction for color development so that only one time point can be obtained from each reaction. Commercial pNPP usually does not have sufficient purity and gives a high background signal. Thus, further purification of pNPP obtained from commercial sources may be necessary. In addition to small molecule substrates, PTP activity can be assayed using phosphopeptide substrates of the PTP and free phosphate release measured using Malachite green/Biomol Green reagents. This has the benefit of potentially identifying compounds that inhibit the PTP activity by binding to the peptide substrate binding site. In chemical library screening, secondary confirmation assays should be performed using different substrates and/or methods to evaluate whether a hit interferes with the assay measurement and is not a true inhibitor of the PTP. Ideally, PTP activity is assayed using a substrate at the concentration that equals its Km. Under this condition, a competitive inhibitor will have an $IC_{50} = 2 \times Ki$.

Assessment of target inhibition in vivo—Shp2 PTP is activated in the cells after EGF stimulation. Therefore, inhibition of Shp2 by a Shp2 inhibitor may be determined directly by immunoprecipitation of Shp2 from cells after stimulation with EGF and the Shp2 PTP activity measured by an appropriate assay. This has been used in our study of NSC-87877, in which we assayed Shp2 inhibition in EGF-stimulated HEK293 and MDA-MB-468 cells [83]. This assay requires that the Shp2 inhibitor binds tightly to Shp2 so that the inhibitor-enzyme complex does not dissociate completely during immunoprecipitation. For an inhibitor such as a competitive inhibitor that does not covalently modify the enzyme, it is difficult to correlate the extent of Shp2 PTP inhibition in the cells by a sub-saturated concentration of the inhibitor with the percentage of Shp2 PTP inhibition in the immune complex PTP assay. This is because the amount of inhibitor dissociated from the immune complex during immunoprecipitation is unknown.

A Shp2 substrate in EGF-stimulated MDA-MB-468 breast cancer cells is paxillin [52]. Paxillin is tyrosine-phosphorylated under serum-starved conditions and is dephosphorylated by Shp2 after EGF-stimulation. The Shp2 inhibitor NSC-87877 has been shown to block EGF-induced paxillin tyrosine dephosphorylation in MDA-MB-468 cells [83]. Similarly, Hellmuth et al found that HGF also induced paxillin dephosphorylation in MDCK cells, which was attenuated by the Shp2 inhibitor PHPS1 [28].

Shp2 mediates Ras-Erk1/2 activation by growth factors. Shp2-mediated signaling pathways may regulate the strength of the peak activity or the length of the activation phase. Growth factor-induced Erk1/2 activation has been used as a surrogate marker for Shp2 inhibition in

EGF- and HGF-stimulated cells by Shp2 inhibitors [28,83]. In HEK293 cells, NSC-87877 inhibited EGF-stimulated Erk1/2 activation with an EC_{50} of 6 μM [83]. In MDCK cells, although PHPS1 did not affect HGF-stimulated phospho-Erk1/2 (pErk1/2) signal at 5 min, it inhibited HGF-stimulated pErk1/2 signal at 15-360 min [28].

Three approaches may be used to rule out the possibility that a Shp2 inhibitor non-specifically blocks Erk1/2 activation. The first is by comparison of Erk1/2 activation by a growth factor and by phorbol ester. While Shp2 mediates EGF- and HGF-induced Erk1/2 activation, the phorbol ester-induced Erk1/2 activation is independent of Shp2. Therefore, phorbol ester-induced Erk1/2 activation should not be affected by inhibition of Shp2 PTP activity. This has been used for determination of specific inhibition of the Shp2-dependent Erk1/2 activation by NSC-87877 and PHPS1 [28,83]. The second approach utilizes active Shp2 constructs that by-pass the upstream signaling steps of receptor tyrosine kinases. Constitutive Erk1/2 activation can be initiated intracellularly using a chimeric protein of Gab1 PH domain and N-SH2 domain deletion Shp2 mutant or using a myristoylated Shp2 [48]. This approach has been used in evaluation of NSC-87877 [83]. In addition, certain cell lines that express the leukemia-associated Shp2E76K mutant also result in constitutive Erk1/2 activation [28,81]. These cell lines may be used to assess Shp2 inhibition by monitoring pErk1/2 as reported [28]. Because Ras is downstream of Shp2, constitutively active Ras mutant-induced Erk1/2 activation by-passes the requirement of Shp2 PTP activity. Thus, cells expressing a constitutively active Ras, such as H-RasV12, is used as an approach to rule out the possibility that a Shp2 inhibitor suppresses Erk1/2 activation by affecting a signaling step downstream of Ras [28].

In addition to these biochemical analyses, cell-based biological responses have also been used to assess Shp2 PTP inhibition in the cells. HGF-induced scattering and branching morphogenesis of MDCK cells is dependent on Shp2 PTP activity [92,93]. HGF-induced MDCK cell scattering was used as a secondary assay in the screening of Shp2 inhibitors by Hellmuth et al [28], while MDCK cell scattering induced by HGF and by a myristoylated, active Shp2 construct was used to examine PHPS1 on inhibition of Shp2 in MDCK cells. The effect of PHPS1 on MDCK cells was phenocopied by Shp2 shRNAs.

In solid tumor-derived cell lines, inhibition of Shp2 with small molecule PTP inhibitors, shRNAs or dominant negative mutants can significantly reduce cell proliferation and soft-agar colony formation, indicating that inhibition of Shp2 can suppress cancer cell proliferation and the transformation phenotype. However, the extent of inhibition varies among different cancer cell lines [28]. Parameters for prediction of sensitivity to Shp2 inhibition in carcinoma cell lines have not been established.

Gain-of-function Shp2 mutants such as Shp2E76K and Shp2Y61D can transform certain cytokine-dependent myeloid cell line [81] and can cause fatal myeloproliferative disease in conditional knockin mice [82]. It is anticipated that these cell lines and animal models will be used in the future for pre-clinical studies of Shp2 PTP inhibitors.

OTHER PTP CANDIDATES OF ANTICANCER DRUG TARGETS

PTP1B

PTP1B (encoded by the *PTPN1* gene) is a Class I non-receptor classical PTP. It contains a PTP domain, two Pro-rich sequences and a hydrophobic endoplasmic reticulum anchor domain at the C-terminal region. PTP1B dephosphorylates insulin receptor, which inhibits insulin signaling and thus contributes to insulin resistance in Type II diabetes. PTP1B was reported to activate c-Src by dephosphorylation of the inhibitory pTyr-530 [94]. PTP1B-mediated Src activation is required for transformation of MCF-10A by ErbB2 (HER2/*Neu*)

[95] and contributes to tumor growth of SW48 colon cancer cells [96]. Another PTP1B substrate is p62Dok, which binds p120RasGAP and is a negative regulator of the Ras-Erk1/2 pathway. PTP1B-deficient fibroblasts have reduced PDGF-induced Ras and Erk1/2 activation but increased p62Dok tyrosine phosphorylation and active Akt [97]. PTP1B activity also promotes cell migration [98].

PTP1B is overexpressed in breast and ovarian cancer, which correlates with ErbB2 overexpression [99,100]. In transgenic mouse models of mammary tumorigenesis, PTP1B deficiency delays tumor onset induced by ErbB2 and resistance to lung metastasis [101,102], whereas overexpression of PTP1B in mammary gland results in development of spontaneous mammary tumors [102]. The effects of PTP1B deficiency in these transgenic mice correlates with reduced Erk activation. These results illustrate PTP1B as a tumor promoter and suggest PTP1B as a therapeutic target in ErbB2-positive breast cancer.

However, tumor suppressor effects of PTP1B have also been observed. PTP1B deficient mice do not have spontaneous tumor incidence. In p53 null mice, PTP1B deficiency renders mice more susceptible to B-cell lymphomas [103], which correlates with decreased apoptosis. PTP1B is a negative regulator of c-Met, Bcr-Abl, and p130Cas. Thus, PTP1B has dual roles in cancer.

Since PTP1B was identified as a target for diabetes and obesity, extensive efforts have been undertaken by industrial and academic laboratories to develop PTP1B PTP inhibitors [6,7]. The discovery of the secondary substrate binding site near the catalytic pocket of the PTP1B has facilitated the development of bidentate inhibitors [6,31,32,47]. Several PTP1B inhibitors with low nanomolar Ki have been identified [6]. Among the most potent and selective PTP1B inhibitor is the bidentate difluoromethylphosphonate containing compound (**11**) (Fig. **(10)**), which has 2.4 nM Ki to PTP1B, > 10-fold selectivity against TCPTP and >600-fold selectivity against other PTPs [47]. However, compound (**11**) is not cell permeable. Cell permeable analogs such as compound (**16**) (Fig. (**11**)) and prodrug strategies have been used to deliver compound (**11**) into cells [104,105]. Four series of difluoromethylphosphonate containing compounds were optimized at Merck Frosst Canada using bioavailability in rodents as a key parameter [106]. Compound (**17**) (Fig. **(11)**) was identified as an orally active PTP1B inhibitor (IC_{50}: 120 nM) with good pharmacokinetic properties. The selectivity of compound (**17**) against other PTPs is unclear except that it does not inhibit CD45 (IC_{50}: >50 μM). Compound (**17**) is able to lower blood glucose level in mice in a dose dependent manner, indicative of PTP1B inhibition in the animals [106]. In an ErbB2-induced mammary tumor mouse model, orally administration of compound (**17**) (30 mg/kg for 21 days) significantly delays the onset of mammary tumor development [102]. The median time to tumor onset was extended from 28 days in the vehicle-treated mice to 75 days in the drug-treated mice. It is possible that compound (**17**) is not specific for PTP1B and the potential cross-inhibition of other PTPs may contribute to its anti-tumor effect. However, the combination of genetic and pharmacologic evidence demonstrates PTP1B as target for intervention of ErbB2-dependent breast cancer.

Although not tested as anti-cancer drugs, two PTP1B inhibitors, Ertiprotanib (**18**) and MSI-1436 (Trodusquemine) (**19**) have progressed to human clinical trials as anti-diabetes and anti-obesity drugs [107,108]. The development of Ertiprotanib was discontinued because of insufficient efficacy and concern of multiple in vivo targets. Trodusquemine is a natural product isolated from the dogfish shark *squalus acanthias* [109]. It suppresses appetite and causes fat-specific weight loss in diet-induced obsese mice [108]. Trodusquemine is in phase I trials.

PRL-3

The phosphatase of regenerating liver (PRL) sub-group of DSPs are ~20 kDa small PTPs with three members PRL-1 (*PTP4A1*), PRL-2 (*PTP4A2*), and PRL-3 (*PTP4A3*) [19,110]. The PTP domain of PRLs is followed by a polybasic region and the CAAX prenylation motif at the C-terminus. The polybasic region facilitates interaction with membrane phospholipids and nuclear localization. PRLs can be farnesylated via the C-terminal CAAX box. Farnesylated PRLs are associated with the plasma membrane and early endosome [111]. PRL-1 has been detected in nuclei.

After the seminal discovery that PRL-3 is overexpressed in metastatic colorectal cancer [112], many laboratories have investigated the roles of PRLs in human cancer. Elevated PRL-3 expression has been detected in metastatic lesions of colorectal cancer regardless the site of metastasis [113]. High levels of PRL-3 expression in primary colorectal cancer correlated with poor prognosis [114]. Up-regulation of PRL-3 has also been reported in primary breast, gastric, liver, ovarian, nasopharyngeal carcinoma, myeloma, and various cancer cell lines [19,115-121]. In tumor xenograft experiments, Zeng et al reported that Chinese hamster ovary (CHO) cells expressing PRL-1 and PRL-3 develop metastatic tumors in mice [122] and that the PTP activity is essential for tumor metastasis [123]. Association of PRL-1 and PRL-2 expression with human cancer has not been thoroughly examined. However, PRL-1 and PRL-2 are expressed in most tissues.

PRLs have been found to regulate RhoA and RhoC [124], down-regulate PTEN [125], control Src expression and activity, and activate Erk1/2 [126,127]. Forte et al reported that PRL-3 dephosphorylates pThr-567 on Ezrin in HCT116 colon cancer cells [128]. In cell cultures, PRL-3 has been shown to promote cell proliferation, migration, invasion, epithelial-mesenchymal transition, and angiogenesis. Therefore, extensive data have shown that PRLs, especially PRL-3, play important roles in metastatic cancer.

The abundant evidence supporting the cancer promoting roles of PRLs has sparked interests in the search for PRL inhibitors. Solution structures of human PRL-3 [129,130] and a crystal structure of human PRL-1 [131] have been determined. The catalytic pockets of PRLs are unusually shallow and wide, indicative of difficult drug targets. Nevertheless, Daouti et al employed a fluorescence polarization assay to screen the Roche chemical library and identified thienopyridone (**20**) (Fig. (**12**)) as a selective PRL inhibitor [132]. Thienopyridone inhibits PRL-3 with an *in vitro* IC_{50} of 0.457 μM and displays > 100-fold selectivity against other PTPs, although the mechanism of inhibition has not been defined. Thienopyridone is cell active and induces $p130^{Cas}$ cleavage and anoikis and inhibits anchorage-independent growth of colon cancer cells.

In other efforts, natural products ginkgetin **(21**) and sciadoptysin **(22**) were identified as weak inhibitors of PRL-3 (IC_{50}: 25.8 and 46.2 μM, respectively) [133]. Twelve lead compounds of PRL-3 inhibitors, with IC_{50} values in the range of 10-50 μM, were identified by virtual screening of an interbioscreen library using the solution structure of PRL-3 [134]. A panel of rhodanine derivatives were evaluated as PRL-3 inhibitors and compound (**23**) was found to inhibit PRL-3 with an IC_{50} value of 0.9 μM [135]. Furthermore, Pathak et al reported that the anti-protozoa drug pentamidine inhibits PTPs including PRL-3 [136].

Given that PRL-3 is frequently up-regulated in various types of metastatic cancer, effort has also been made to suppress PRL-3 expression. While the molecular target is unknown, curcumin, a polyphenol component of the spice turmeric, has been found to downregulate PRL-3 mRNA in a manner independent of p53 [137]. While this does not represent a traditional approach to drug discovery of molecular cancer targets, it highlights the usefulness of compounds derived from natural products.

CDC14

Cdc14 is an evolutionally conserved DSP. In budding yeast, Cdc14 is required for mitosis exist by inactivating mitotic Cdk through dephosphorylation of Cdk substrates Cdh1 and Sic1 [138]. Mammalian cells have two Cdc14, Cdc14A and Cdc14B. Cdc14s are Pro-directed phosphatases that dephosphorylate specific phosphorylation sites within the pSer/pThr-Pro motif, which are substrate sites of Pro-directed kinases Cdks or MAP kinases. Cdc14B has a nuclear targeting sequence in the N-terminal region. Besides the PTP domain, Cdc14s have non-conserved C-terminal regions. Cdc14A and Cdc14B are differently regulated in mammalian cells. Cdc14A is localized at the interphase centrosome and at the spindle midzone. Cdc14B is localized at nucleoli during interphase and at the mitotic spindle during mitosis. Overexpression of Cdc14A results in premature centrosome splitting and multi-polar spindles [139], suggesting that over-reactive Cdc14 contributes to genomic instability. Down regulation of Cdc14A leads to centrosome separation and cytokinesis defects and induces cell death [139]. Cdc14A is up-regulated in human cancer cell lines that contain p53 mutants [140]. Although the critical Cdc14 substrates that regulate centrosome separation and cytokinesis in mammalian cells remain to be identified, a few Cdc14 substrates have been reported. These include RN-tre, Cdc25A and p53 [140-142].

No Cdc14 inhibitor discovery effort has been reported. The structure of Cdc14 PTP catalytic domain is consisted of tandem A- and B-DSP domains, in which the PTP signature motif $C(X)_5R$ is located in the B-domain. [143]. The catalytic site is located in a narrow, long groove formed at the interface of the A- and B-domains. Three acidic residues are located at one end of the substrate binding groove. These interesting features of the Cdc14 catalytic site are attractive characteristics of a potentially useful drug target.

LMW-PTP

Low molecular weight PTPs (LMW-PTPs) are encoded by the *ACP1* gene. *ACP1* is the sole Class II Cys-based PTP gene in human [144]. LMW-PTPs are ~18-kDa and have the conserved Cys-based PTP signature motif $C(X)_5R$ but little sequence homology with other PTPs. Four isoforms are produced by alternative splicing. Only isoforms 1 and 2 (IF1 and IF2) have PTP activity. While IF1 and IF2 differ in *in vitro* enzyme kinetics properties [145], their functional difference in the cells is unclear. A number of substrates of LMW-PTP have been reported that include EphA2, FAK, PDGFR, insulin receptor, ZAP-70, and STAT5. It has been reported that upregulation of LMW-PTP in colon cancer and neuroblastoma correlates with unfavorable outcome [146]. Overexpression of LMW-PTP is sufficient to transform MCF10A breast epithelial cells [20]. Overexpression of LMW-PTP in NIH3T3 cells causes early onset and larger tumors in nude mice while the catalytic inactive LMW-PTP inhibit NIH3T3 tumor growth [21]. Cell transformation and tumor promoting activities of LMW-PTP are associated with dephosphorylation of the EphA2 receptor tyrosine kinase [20,21].

Two natural products morin and ferruginol are reported to weakly inhibit LMW-PTP [147,148]. In an effort to optimize flavonoid-based LMW-PTP inhibitors, Forghieri et al identified several compounds having sub-micromolar to low micromolar IC_{50} to LMW-PTP, although these compounds display similar inhibitory activity to PTP1B [149]. In another effort, Maccari et al characterized a series of compounds based on the 4-[(5-aryldene-2,4-dioxothanolidin-3-yl)methyl]benzoic acid scaffold and identify several potent LMW-PTP inhibitors having sub-micromolar IC_{50} values [150]. Again, these compounds cross-inhibit PTP1B.

Cdc25

Cdc25 are class III Cys-based PTPs. Three isoforms are present in mammals, Cdc25A, Cdc25B, and Cdc25C. They are dual specificity phosphatases (DSPs) that activate cyclin-dependent kinases (Cdks) by dephosphorylation of the inhibitory dual Thr-Tyr phosphorylation sites (Thr-14 and Tyr-15 on Cdk1) in the N-terminal area of Cdks [151] (Fig. (**13**)). Cdc25s are positive regulators of cell cycle progression and also play a central role in the checkpoint response to DNA damage. Cdc25 expression and activity are subject to transcriptional and posttranslational regulation. Cdc25 overexpression has been reported in many types of human cancer and is correlated with higher grade tumors and poor disease-free survival. Although Cdc25 overexpression is not sufficient to cause cancer, overexpression of Cdc25A reduces the latency of MMTV-H-ras induces mammary tumors in mice and gives rise to more invasive mammary tumors in MMTV-Neu mice. [152]. Homozygous Cdc25A knockout is embryonic lethal in mice. In heterozygous Cdc25A knockout mice, the latencies of MMTV-Ras and MMTV-Neu evoked mammary tumors are significantly delayed [153]. $Cdc25A^{+/-}$ mouse embryonic fibroblasts are resistant to transformation by co-expression of H-RasV12 and a dominant-negative p53 and are more responsive to radiation-induced cell cycle arrest. These findings suggest that Cdc25 overexpression contributes to tumorigenesis. Interestingly, Cdc25C is located in chromosome 5q, which is deleted in a sub-group of MDS patients. MDS patients with 5q deletion are responsive to lenalidomide treatment, which has been attributed to the haplodeficiency of Cdc25C [154]. It will be important to test if a Cdc25 inhibitor can sensitize non-5q deletion MDS cells to lenalidomide.

Cdc25s have been targeted for novel anticancer drug discovery for more than a decade. Many Cdc25 inhibitors have been reported. These Cdc25 inhibitors belong to six classes of small molecules as summarized by Lazo and Wipf [12] that include natural products, lipophilic acids, quinones, electrophiles, phosphate mimics, peptide analogs. In contrast to PTP1B inhibitor discovery efforts in which phosphotyrosine mimetics are frequently used in the inhibitor design, redox and microreactive compounds are frequently identified among Cdc25 PTP inhibitors. Many of these reactive Cdc25 inhibitors, such as NSC-663284 (**24**), Fig. (**14**), have IC_{50} values in the sub-micromolar range. The most potent Cdc25 inhibitor is the bis heterocyclic quinone IRC-083864 (**25**) [155]. IRC-083864 inhibits Cdc25s with IC_{50} values in the range of ~20 nM but does not significantly inhibit other PTPs tested at 50 nM. IRC-083864 and two other less potent Cdc25 PTP inhibitors, BN82002 (**26**) and BN82685 (**27**), have been shown to inhibit tumor xenograft growth in mice [155-158].

Eya

Eyes Absents (*EYAs*) are Asp-based PTPs that include four members Eya1, Eya2, Eya3 and Eya4. Eya is a component of the SIX-EYA-DACH transcription network [159-161]. Eya2 is overexpressed in ovarian carcinoma and promotes tumor xenograft growth [162]. Recently, Eya has been identified as the histone H2A.X PTP, serving as the switch between DNA-damage repair and apoptosis after DNA double-strand breaks (DSBs). Tyr-142 at the C-terminus of histone H2A.X is constitutively phosphorylated under unstressed conditions by the WSTF tyrosine kinase [22,23]. After DNA DSBs, ATM/ATR phosphorylates H2A.X at Ser-139 that generates γ-H2A.X and phosphorylates Eya3 at Ser-219 that results in localization of Eya1/Eya3 complex to γ-H2A.X and dephosphorylation of Tyr-142. Tyr-142 dephosphorylation by Eya1/Eya3 is necessary for recruitment of repairs factors via binding of DNA damage checkpoint protein MDC1 to the pSer-139 site of γ-H2A.X for repairing damaged DNA. While inhibiting MDC1 binding to pSer-139, Tyr-142 phosphorylated γ-H2A.X recruits JNK1 to initiate pro-apoptotic program. Thus, blocking Tyr-142 dephosphorylation by inhibition of Eya prevents DNA damage repair and promotes apoptosis [23,24]. Conceivably, inhibition of Eya PTP activity may be used to enhance the

therapeutic efficacy of DNA damage-based cancer therapy, such as radiation and certain chemotherapy agents.

A structure of Eya2 PTP domain shows that five conserved Asp and five conserved Glu are located on the surface around the active site [24]. The highly negative-charged active site is consistent with the finding that Eya recognizes the basic substrate histone H2A.X. While no Eya PTP inhibitor discovery effort has been reported, the recent realization of the critical role of Eya in DNA damage repair and apoptosis is likely to stimulate the quest for novel Eya PTP inhibitors.

CONCLUDING REMARK

Protein phosphatases represent 4% of the druggable genome [163]. It is now realized that specific PTPs can function as oncogenes or non-oncogene addiction genes that contribute positively to the initiation and maintenance of human cancer. Potential PTP targets for novel anticancer drug discovery are identified in all four classes of PTPs. While catalytic pockets of many PTPs are considered challenging for drug discovery efforts, appropriate surface properties surrounding PTP catalytic sites do exist that allow development of potent and selective inhibitors for many PTPs. This notion has been supported by the identification of a number of highly potent and selective inhibitors to specific PTPs.

Although progress has been made, the field of PTP inhibitor development is in its infancy. Major efforts are required by academic institutions and industries to overcome various hurdles in order to successfully develop the first PTP inhibitor as a drug. Target identification and validation in vitro and in vivo, lead discovery and optimization, identification of specific substrates in vivo and surrogate markers for assessment of target inhibition, pharmacologic profiling and selective toxicity analysis all require great efforts. The realization that PTPs do not simply counter the function of PTKs, but could cooperate with PTKs to promote oncogenesis should attract great interests in developing PTP inhibitors for cancer therapy in the coming years.

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ABBREVIATIONS

AML	acute myelogenous leukemia
BTAMs	bisphosphoryl tyrosine-based activation motifs
Cdk	cyclin dependant kinase
CML	chronic myeloid leukemia
csw	corkscrew
DiFMUP	6,8-difluoro-4-methylumbelliferyl phosphate
DSB	double strand break
DUSP/DSP	dual specificity phosphatase
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
Erk1/2	extracellular signal regulated kinase 1 and 2

Eyas	eyes absents
FAK	focal adhesion kinase
FDA	Food and drug administration
FGFR	fibroblast growth factor receptor
FRS2α	Fibroblast growth factor receptor substrate 2α
GAP	GTPase activating protein
GM-CSF	granulocyte macrophage-colony stimulating factor
IFN-γ	interferon gamma
IL	interleukin
IRS-1	insulin receptor substrate-1
ITIM	immunoreceptor tyrosine-based inhibitor motif
JMML	juvenile myelomonocytic leukemia
LMW	low molecular weight
MAP	mitogen-activated protein
MDS	myelodysplastic syndrome
MPD	myeloproliferative disorder
MPZL1	myelin protein zero-like-1
OTAP	organic anion transporter
PDGFR	platelet-derived growth factor receptor
PECAM-1	platelet endothelial cell adhesion molecule
PH	pleckstrin homology
PHPS	phenylhydrazonopyrazolone sulfonate
pNPP	p-nitrophenyl phosphate
PRL	phosphatase of regenerating liver
PTEN	phosphatase and tensin homolog 10
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
pY	phosphotyrosine
SFK	Src family kinase
SH2	src-homology-2
SHPS1	Shp substrate-1
SIRP	signal regulatory protein
STAT	signal transduction activator of transcription

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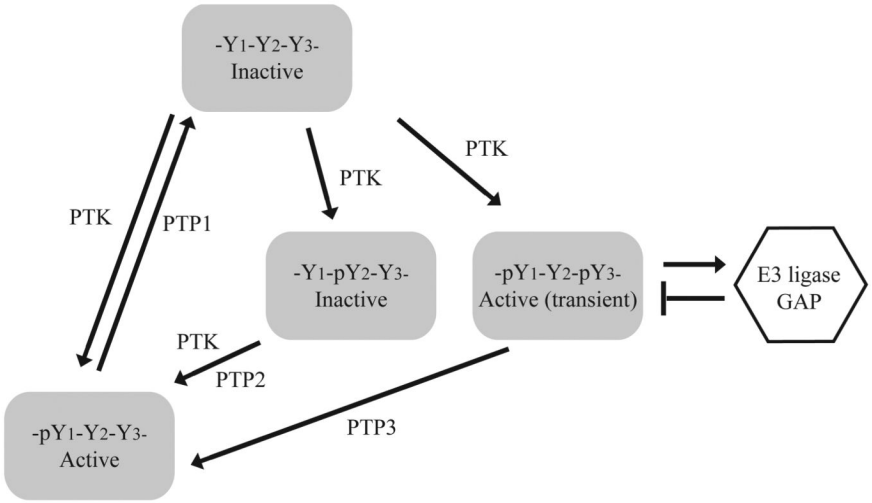

Fig. (1).

Positive and negative roles of tyrosine phosphorylation in cell signaling. In this illustration, three tyrosine residues (Y_1, Y_2, Y_3) on a protein may be subject to phosphorylation by a PTK. Phosphorylation of Y_1 increases the activity of the protein. Phosphorylation of Y_2 inhibits the activity of the protein. Phosphorylation of Y_3 induces feedback inhibition such as recruitment of E3 ligase that causes degradation of the protein or GTPase Activator Protein (GAP) that turns off G-proteins. While dephosphorylation of Y_1 by PTP1 inactivate the protein, dephosphorylation of Y_2 and Y_3 by PTP2 and PTP3 are necessary for sustained activity of the protein. Thus, PTP1 is a negative regulator whereas PTP2 and PTP3 are positive regulators that coordinately control the activity of the protein.

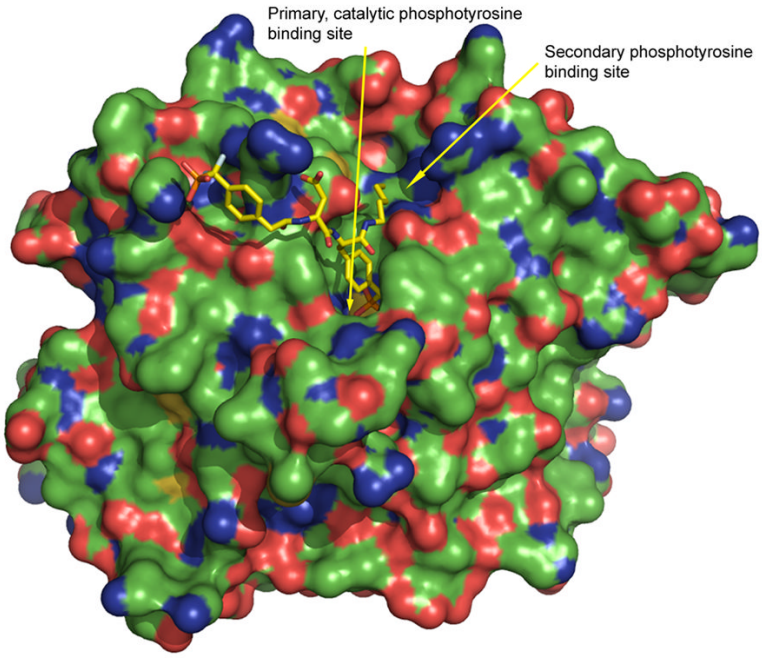

Fig. (2).
Illustration of the secondary phosphotyrosine binding site of PTP1B (from pdb code 1PXH), colored by element: carbon, green; oxygen, red; nitrogen blue, sulfur, yellow.

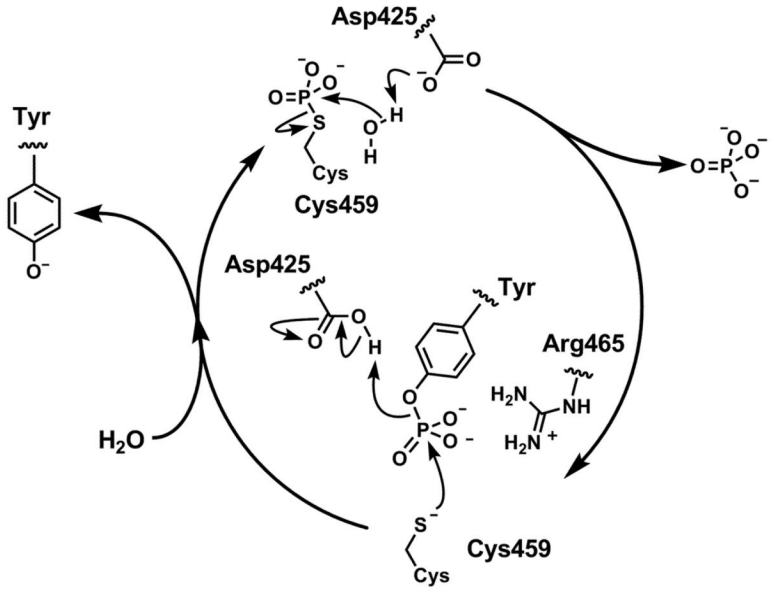

Fig. (3).

General mechanism of tyrosine dephosphorylation using Shp2 residue numbering. The nucleophylic cysteine residue Cys459, part of the signature PTP loop sequence (residues 457-467), VHCSAGIGRTG, attacks the phosphorus atom to break the PO bond in an S_N2-like fashion. The aspartic acid residue Asp 425, that forms part of the WPD loop, acts as the proton donor to protonate the tyrosine phenol. This generates a labile thiophosphate intermediate that is subsequently hydrolysed to liberate the free cysteine residue. The aspartic acid plays a role as a general base, by abstracting a proton from the water molecule. The phosphotyrosine binding site of PTPs is generally populated with positively charged residues to stabilize the signature, in particular the arginine residue Arg465 of the P loop is important in stabilizing the negative charge of the pentavalent phosphorus formed upon initial addition of the cysteine to the phosphotyrosine group.

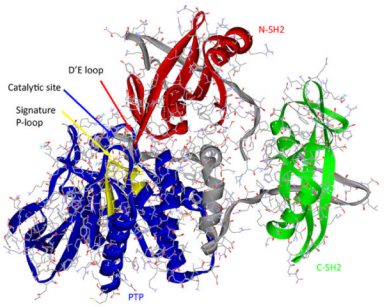

Fig. (4).

X-ray crystal structure of Shp2 (pdb code 2SHP) showing the N-SH2 (red), C-SH2 (green) and PTP (blue) domains. The two linking sequences that join these three domains are shown in gray. The signature P-loop motif (VHCSAGIGRTG) is illustrated in yellow.

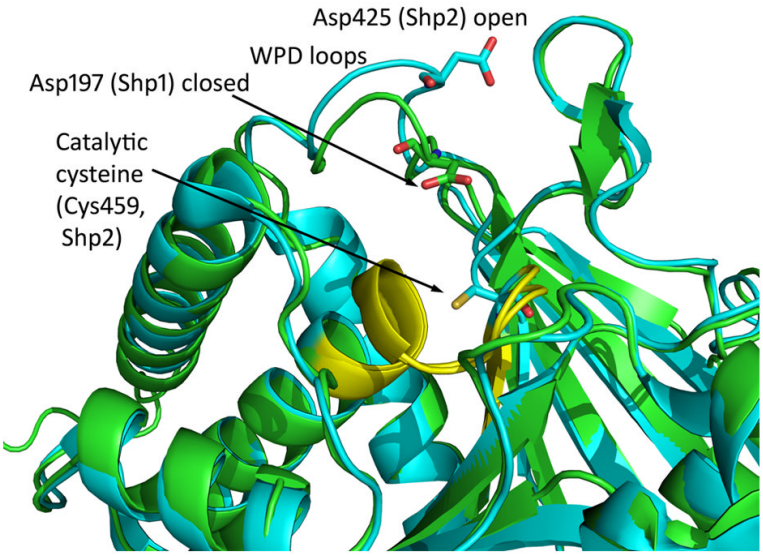

Fig. (5).
Overlay of the X-ray crystal structures of the PTP domain of Shp1 (pdb 1GWZ, colored green) and Shp2 (pdb 3B7O) colored blue, clearly indicating the closer proximity of the WPD aspartic acid residue to the catalytic cysteine residue for Shp1 as a consequence f the closed WPD loop conformation.

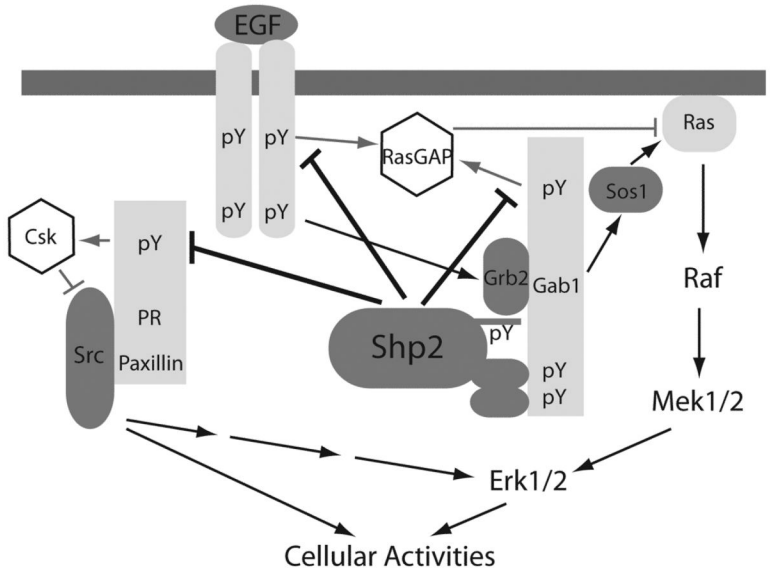

Fig. (6).

Signaling pathways from the EGF receptor to Erk1/2 and Src in adherent cells. Representative mechanisms are shown to illustrate that Shp2 activates Src family kinases by preventing recruitment of Csk to Src complexes and activates Ras-Erk1/2 by regulating RasGAP and Src.

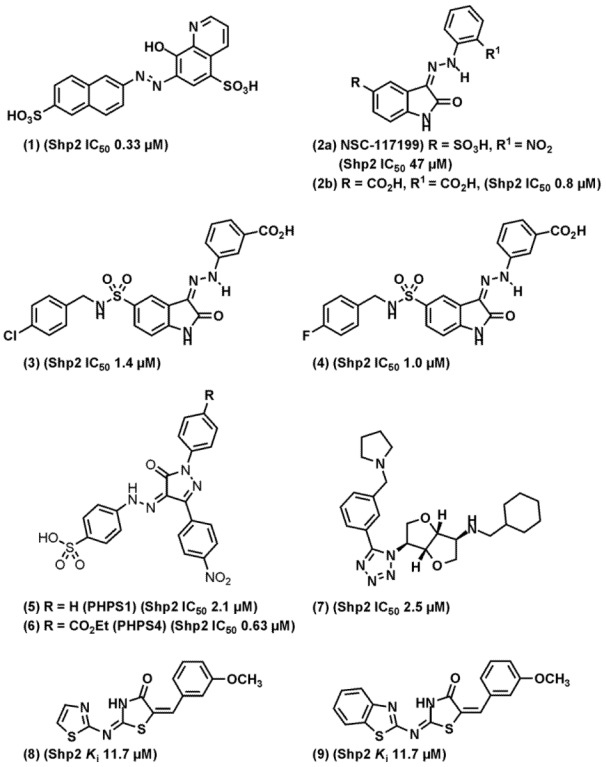

Fig. (7).

Representative of Shp2 inhibitors. (**1**), NSC-87877. (**3**) and (**4**) are synthesized based on screening hit (**2**). (**5**) and (**6**), PHPS1 and PHPS4.

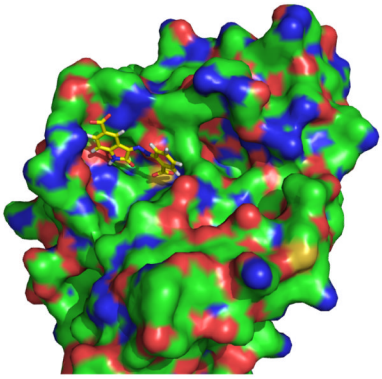

Fig. (8).
Docked structure of isatin (**2b**) with Shp2 (derived from structure pdb code 2SHP), colored by element: carbon, green; oxygen, red; nitrogen blue, sulfur, yellow: hydrogens removed from the protein for clarity.

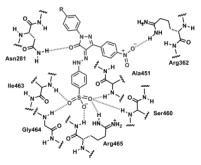

Fig. (9).

Proposed schematic docking model of PHPS1 (**5**) with the catalytic site of Shp2. Hydrogen bonds between the ligand and phosphatase as predicted by MOE v2006.3 are shown as hatched lines.

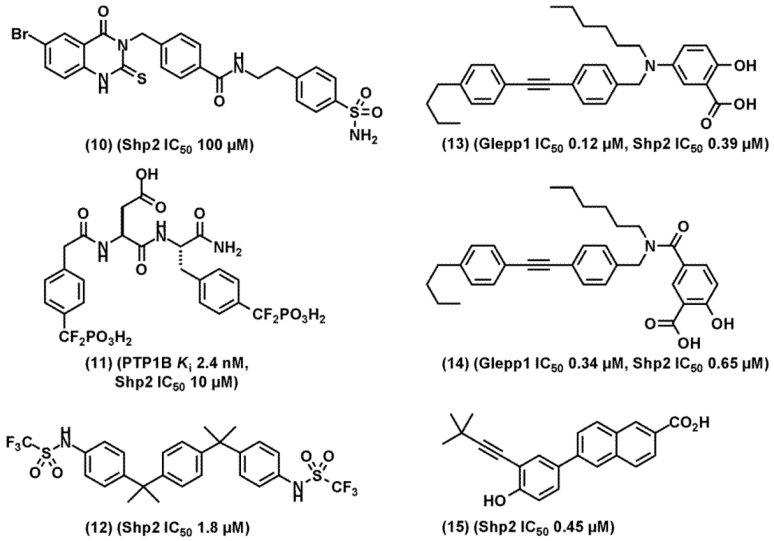

Fig. (10).

Shp2 inhibitors and other PTP inhibitors that cross-inhibit Shp2. (**10**), an Shp2 selective inhibitor identified from virtual screening of secondary binding site. (**11**), a potent PTP1B that cross inhibits Shp2. (**13**) and (**14**), orally bioavailable Glepp1 inhibitors that cross-inhibits Shp2. (**15**), a nuclear receptor small heterodimer partner that cross inhibits Shp2.

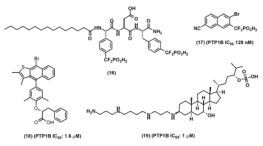

Fig. (11).

Representative of PTP1B inhibitors. (**16**), a cell permeable analog of compound (**11**). (**17**), an orally bioavailable PTP1B inhibitor. (**18**), Ertiprotafib. (**19**) MSI-1436 (Trodusquemine)

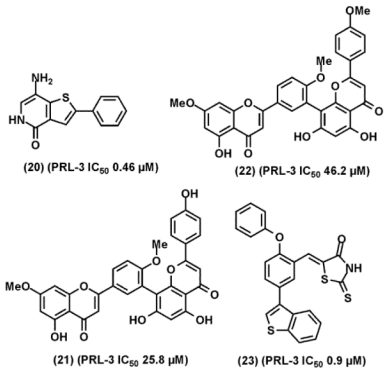

Fig. (12).

Representative of PRL-3 inhibitors. (**20**), thienopyridone. (**21**), ginkgetin. (**22**), sciadoptysin. (**23**), a rhodanine derivative.

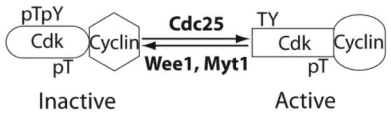

Fig. (13).

Cdc25 dual specificity phosphatases are positive regulators of Cdks. The activity of a Cdk is regulated by three phosphorylation sites Thr14, Tyr15, and Thr161 (residues numbers based on Cdk1). Thr14 and Tyr15 phosphorylation catalyzed by Wee1 and Myt1 kinases are inactivating while Thr161 phosphorylation by Cak is activating. Cdc25 phosphatases dephosphorylate Thr14 and Tyr15, resulting in Cdk activation.

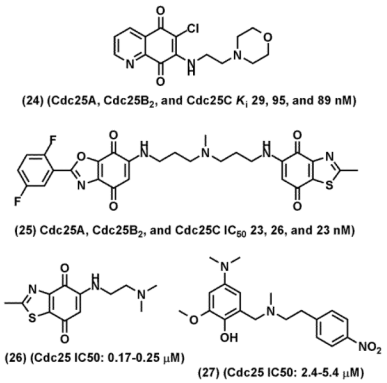

Fig. (14).

Representative of Cdc25 inhibitors. (**24**), NSC-663284. (**25**), IRC-083864, (**26**), BN82002, (**27**) BN82685.

Table 1

PTP inhibitors that have been evaluated *in vivo*

Inhibitor	PTP	Animal Studies	Human Trials	Firm	Reference
BN82002 Cmp **26***	Cdc25	Inhibit Mia PaCa-2 pancreatic tumor xenograft growth.		IPSEN	[156]
BN82685 Cmp **27**	Cdc25	Inhibit Mia PaCa-2 pancreatic tumor xenograft growth.		IPSEN	[157]
IRC-083864 Debio-0931 Cmp **25**	Cdc25	Inhibit Mia PaCa-2 pancreatic tumor and LNCaP prostate tumor xenograft growth.	Phase I (planned)	IPSEN/ Debiopharm	[155]
Cmp **17**	PTP1B	Lower blood glucose level. Delay onset of ErbB2-induced mammary tumor.		Merck Frosst	[102]
Ertiprotafib Cmp **18**	PTP1B	Lower fasting blood glucose and insulin. Improve glycemic excursion. Improve lipid profiles.	Phase II in type II diabetes (Discontinued because of insufficient efficacy and concerns about side-effects due to inhibition of other targets)	Wyeth Research	[107]
MSI-1436 Trodusquemine Cmp **19**	PTP1B	Suppress appetite Induce fat-specific weight loss Improve insulin and leptin levels	Phase I	Genaera	[108]
Cmp **13**	Glepp1	Inhibit thioglycolate-induced peritonitis. Inhibit dermatitis. Inhibit ulcerative colitis.		Merck Serono	[90]

Cmp: compound