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Integrating virtual and biochemical screening for protein tyrosine phosphatase inhibitor discovery ☆

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ABSTRACT

Protein tyrosine phosphatases (PTPs) represent an important class of enzymes that mediate signal transduction and control diverse aspects of cell behavior. The importance of their activity is exemplified by their significant contribution to disease etiology with over half of all human PTP genes implicated in at least one disease. Small molecule inhibitors targeting individual PTPs are important biological tools, and are needed to fully characterize the function of these enzymes. Moreover, potent and selective PTP inhibitors hold the promise to transform the treatment of many diseases. While numerous methods exist to develop PTP-directed small molecules, we have found that complimentary use of both virtual (*in silico*) and biochemical (*in vitro*) screening approaches expedite compound identification and drug development. Here, we summarize methods pertinent to our work and others. Focusing on specific challenges and successes we have experienced, we discuss the considerable caution that must be taken to avoid enrichment of inhibitors that function by non-selective oxidation. We also discuss the utility of using "open" PTP structures to identify active-site directed compounds, a rather unconventional choice for virtual screening. When integrated closely, virtual and biochemical screening can be used in a productive workflow to identify small molecules targeting PTPs.

1. Introduction

1.1. The PTP family

Estimates predict that 30% of the human proteome is subject to phosphorylation [1]. While tyrosine phosphorylation constitutes <0.1% of total phosphorylation in mammalian cells, it represents a critical regulatory mechanism in signal transduction. Balanced signaling is achieved through the exquisite coordination of protein tyrosine kinases (PTKs) and phosphatases (PTPs), which catalyze the phosphorylation and dephosphorylation of diverse substrates, respectively [2,3]. While the active role of PTKs in signaling has long been accepted, PTPs were originally associated with housekeeping functions and their active and direct role in signaling was initially underappreciated [4]. This connotation has since been dismissed by decades of research revealing dynamic regulation, substrate specificity, and activity of the large family of PTPs.

The PTP superfamily consists of over 100 enzymes that can be classified by catalytic mechanism, substrate specificity, and sequence similarity. There are 38 members of the classic PTP family, which harbor strict specificity for tyrosine residues [5]. These enzymes are typified by deep and narrow catalytic grooves that accommodate large aromatic phosphotyrosine rings, while occluding shorter phosphoserine or threonine residues from the base of the active site [6]. Catalysis is initiated when the phosphate group of a substrate extends to the base of the active site and is attacked by a nucleophilic cysteine within a conserved phosphate-binding loop, or P-loop (hallmarked by a H/V-CX_5R-S/T motif). Binding is accompanied by closure of a flexible WPD loop (named for conserved tryptophan, proline, and aspartic acid residues) around the substrate, positioning the invariant aspartate to protonate the oxygen leaving group of the tyrosyl substrate. Finally, a conserved Q-loop coordinates a water molecule and the aspartate of the WPD loop, which catalyze the scission of the phospho-enzyme intermediate complex. This restores the enzyme to its initial state, while producing a free phosphate group and a dephosphorylated

Abbreviations: PTP, protein tyrosine phosphatase; RCC, redox cycling compounds; ROS, reactive oxygen species; HTS, high-throughput screen; PDB, protein data bank; pNPP, para-nitrophenyl phosphate; VS, virtual screening; DTT, dithiothreitol; pTyr, phosphotyrosine.

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substrate [7]. Despite sharing this highly conserved mechanism, PTPs show specificity for a diverse set of substrates that regulate a wide variety of cellular and molecular pathways.

When the normal function of PTPs are dysregulated (by altered expression or activity), they contribute to the aberrant signaling that drives pathological phenotypes of many human diseases [2–4]. In fact, roughly half of all PTP genes have been implicated in at least one human disease to date [1]. The most widely studied PTP in the context of human disease is PTP1B (*PTPN1*), the first member of this class to be purified and characterized [8,9]. Loss of PTP1B promotes insulin and leptin signaling, and has been shown to combat diabetes and obesity in animal models [10–13]. In addition, mutations and SNPs involving PTP1B have been linked to type 2 diabetes [14–18]. In total, over 20 PTPs have been associated with hereditary human diseases, notably SHP2 (*PTPN11*) which is mutated in both Noonan and Leopard syndromes [19,20]. Variants in Lyp phosphatase (*PTPN22*) have been strongly linked to autoimmune diseases, including type I diabetes, rheumatoid arthritis, Graves' disease, and systemic lupus erythematosus [21,22]. Furthermore, PTPs have been linked extensively to cancer, including the identification of 22 PTPs in chromosomal regions frequently amplified or deleted in cancer [23]. Point mutations and epigenetic silencing have also been found to alter PTP expression and activity in numerous cancer types (reviewed in [24]). While many alterations are consistent with tumor suppressive functions, for example those associated with PTEN and PTPδ (*PTPRD*), PTPs that promote cancer initiation and progression have also been discovered [24]. Of note, oncogenic mutations drive SHP2 activation in many hematological cancers, as well as breast cancer and neuroblastoma [25,26]. PRL3 (*PTP4A3*) has been found overexpressed in colorectal cancer metastases, an observation attributed to the positive regulation of cell motility and invasion by the PRL phosphatases [24,27–29]. Collectively, PTPs with positive roles in cancer and other diseases have garnered particular interest as drug targets, with inhibitors to PTP1B, SHP2, Lyp, and others being actively pursued [30–32].

In addition to the dysregulation of PTPs in disease, their physiological functions can also make them attractive as therapeutic targets. An important example of interest to us is PTPσ (*PTPRS*) [33,34]. PTPσ is enriched in the brain where it controls axon guidance and neurite outgrowth [35–38]. Recently, PTPσ was shown to interact with chondroitin sulfate proteoglycans (CSPGs), released at the site of spinal cord injury, and profoundly suppress neural regeneration [39,40]. Thus, the ability to therapeutically block this PTPσ-mediated activity has the potential to improve recovery following spinal cord, and other nervous system injuries.

Taken together, PTPs serve critical functions in normal physiology and actively drive disease phenotypes when their activity or expression is altered. As such, they represent important molecular targets for basic research and drug development. Selectively targeted small molecules are essential tools to interrogate the function of individual PTPs, offering unique advantages of reversibility (in the case of competitive inhibitors), as well as temporal and dose control. In addition to their use as biological tools, small molecules directed to PTPs hold considerable promise as potential therapeutics and innovative approaches chemically targeting phosphatases would certainly unlock a critical class of enzymes for disease modification.

1.2. Challenges in chemical targeting of PTPs

Confounding the fact that PTPs have not been always been accepted as drivers of cell signaling and disease, molecular targeting of these enzymes has been slowed by their perceived "undruggability" [41,42]. First, the functional role of PTPs poses a challenge for drug targeting. Within a single pathway, multiple PTPs may serve important roles, so targeting just one may not elicit the desired effect. Conversely, a single PTP may serve several distinct functions in complex signaling networks. In this case, selectively targeting a single enzyme may elicit several off target and possibly undesired cellular consequences.

Second, and more problematic, there are several chemical properties of PTPs that render drug targeting extremely challenging. The PTP family is characterized by an exceptionally high degree of sequence conservation across their active sites. This sequence similarity accompanies several highly conserved physical domains in and surrounding the active site [5]. This common sequence and structure makes building selectivity into small molecules quite challenging. In addition, the chemical environment of the active site, which so elegantly permits phosphatase activity, has impaired drug discovery efforts. The PTP active site is positively charged, which facilitates its interaction with phosphotyrosine substrates. Unfortunately, this environment also attracts negatively charged molecules with high affinity in drug screening initiatives. Generally, such polar compounds represent undesirable drugs owing to their poor membrane permeability and limited oral bioavailability [31]. In addition, PTP active sites must be maintained in a reduced state to preserve the catalytic activity of the nucleophilic cysteine residue. Consequently, they are extremely susceptible to oxidation. Molecules that support oxidation, such as redox cycling compounds (RCCs), are commonly identified in drug screening initiatives [43]. Because these oxidizing agents will elicit pleiotropic effects on many targets and cellular processes, they do not represent promising selective PTP-directed compounds.

Despite these issues, the important biological and disease roles that PTPs play provides rationale to pursue drug discovery initiatives. In this report, we discuss the integration of both biochemical and *in silico*, or virtual, screening approaches to develop PTP-directed inhibitors. We focus on our recent identification of small molecule inhibitors of PTPσ, highlighting challenges and considerations that arose from that work, while reviewing related efforts for other PTPs. While diverse targeting approaches exist, we specifically discuss methods to find active-site directed small molecules predicted to function as competitive inhibitors.

2. Virtual and biochemical approaches to identify PTP inhibitors

A number of useful methods are available to develop small molecules directed to PTPs (Fig. 1) [41,44]. Most strategies can be classified into one of the following: (1) rational design of inhibitors from substrate-like molecules or molecules with known activity against PTPs; and (2) broad screen of chemical libraries to identify scaffolds that bind and inhibit a PTP of interest. For the former, a substrate-like template mimicking phosphotyrosine [45–49], or a molecule previously shown to bind and inhibit the PTP active site [50–55], is used as a non-selective template while potency and selectivity are improved through chemical modification. A useful resource for this type of initiative is the human phosphatase-substrate network recently developed by Li et al. [56]. In this work, phosphatases have been classified according to their structures and information about known substrates and functions collated. This can aid in the identification of substrate-based templates for chemical development, as well as identify closely and distantly related phosphatases for selectivity evaluation. In this type of approach, small collections of chemicals sharing similarities with these templates can be designed and screened *in silico* or *in vitro* [45,48–51,53,54]. Additionally, when target structures are available, molecular docking studies can be used to rationally design lead molecules with desired properties [46,47,55,57–59]. This type of methodology has been effective in the iterative improvement of inhibitors of PTP1B, YopH, Cdc25, and others.