Selecting protein tyrosine phosphatases as drug targets

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Protein tyrosine phosphatases (PTPs) have emerged as a new and promising class of signaling targets, since the discovery of PTP1B as a major drug target for diabetes and obesity. Blocking individual PTPs results in the activation of specific tyrosine phosphorylation events, but matching PTPs with such pathways and therapeutic indications is a complex undertaking. The history of PTP1B shows that its unusual knockout phenotype and observations with generic and antisense inhibitors *in vivo*, but not its classical molecular biology, triggered the rapid development of inhibitors that are today being developed for the clinic.

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▼ Most intracellular signaling takes place via cascades of phosphorylating enzymes (kinases). The de-phosphorylation reactions are catalyzed by protein phosphatases. Phosphatases can be classified as serine–threonine phosphatases, 'classical' tyrosine phosphatases and dual-specificity phosphatases, which can dephosphorylate all three phosphoamino acids.

The family of tyrosine protein phosphatases

The classical and dual-specficity phosphatase families are commonly referred to as protein tyrosine phosphatases (PTPs). Many serine–threonine phosphorylation cascades are stress-induced pathways involving, among others, mitogen-activated protein kinase (MAPK), Jun-kinase (JNK) and inhibitor of NF-κB (IKKB) kinases that activate transcription factors such as AP-1 (activator protein 1; fos–jun) and NF-κB, whereas tyrosine phosphorylation is associated with cytokine- and growth-factor-induced pathways. Since the discovery of the first PTP [1], many other family members have been identified, and the mammalian gene family is now known to include 90–100 members, as defined by their common ~250 amino acid catalytic domain and/or PTP signature motif ($[H/V]C(X)_5R[S/T]$) [2,3]. The subfamily of classical PTPs is schematically represented in Fig. 1.

PTPs have a highly dynamic role in intracellular signaling (Fig. 2). Blocking PTPs with non-specific inhibitors results, within minutes, in the massive and rapid stimulation of kinase-catalyzed phosphorylation cascades. These observations (large gene family size, dynamic behavior) have led to the assumption that blocking individual PTPs could result in the stimulation of specific pathways. Consequently, interest in PTPs as drug targets in signaling is rapidly increasing [4,5]. The challenge today is to identify PTPs that are crucial for given pathways and to discover the disease for which activation of the pathway can have therapeutic use.

PTP1B as a drug target in diabetes and obesity

Present development status

The current paradigm for PTPs as effective drug targets is PTP1B, following the discovery that mice in which this PTP is deleted are healthy but lean and obesity-resistant, and have reduced plasma levels of insulin and glucose [6,7]. This phenotype was ascribed to enhanced signaling through the insulin receptor and insulin receptor substrate-1 (IRS1), in the absence of PTP1B [5]. The same two groups that have produced the knockout (KO) mouse strains recently also reported that the mutant mice and derived cell lines display enhanced signaling through the leptin receptor [8,9]. These observations establish PTP1B as a therapeutic target for obesity and diabetes, which are linked diseases. Several companies are pursuing the development of PTP1B inhibitors as drugs; among these, Wyeth (http://www.wyeth.com) began testing Ertiprotafib/PTP112 in Phase II trials in March 2001, but reported in June 2002 that it had discontinued

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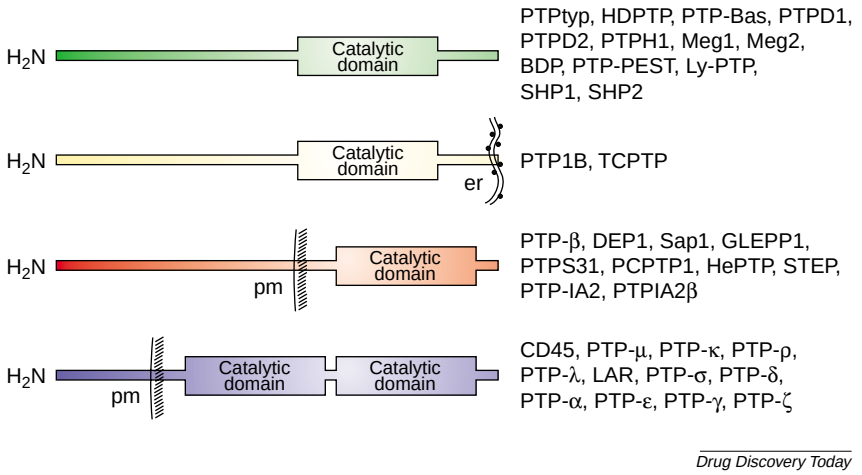


Figure 1. Schematic overview of 'classical' protein tyrosine phosphatases (PTPs). Abbreviations: er, endoplasmic reticulum; GLEPP, glomerular epithelial protein-1 precursor; pm, plasma membrane; SHP, Src homology 2-containing tyrosine phosphatase.

the trials because of unsatisfactory efficacy, as well as the occurrence of dose-limiting side effects among several trial participants. Whether these problems stem from this particular chemical series, species-related differences in PTP1B function, or limited selectivity of the inhibitor for different PTPs is not yet clear. Merck (http://www.merck.com) is developing a PTP1B antisense drug (ISIS-113715). In animal studies, PTP1B antisense reagents partially restored insulin sensitivity in genetically obese rodents [10]. Figure 3 shows a list of companies that are involved in PTP1B-inhibitor discovery, and representative structures that have been disclosed.

PTP1B appears to be an exemplary target for obesity and diabetes. Today, the obesity epidemic is an important factor in the rise of diabetes, and a major drawback for most of today's diabetes drugs, such as peroxisome proliferator-activated receptor (PPAR)-γ agonists and Metformin™ (http://www.bms.com), is that they (further) increase body weight. Therefore, a drug that targets both obesity and hyperglycemia has enormous potential. It is somewhat surprising that a single PTP would control both leptin and insulin signaling. We, and at least two other companies that are developing inhibitors, have found evidence that PTP1B is an allosteric enzyme (unpublished results), which is possibly related to its two P-Tyr binding sites [11], and it could be speculated that the enzyme has a yet to be discovered physiological inhibitor. Vanadate-based inhibitors, which inhibit PTPs non-selectively, have been used with some success in clinical trials with only moderate toxicity. This suggests that a PTP1B inhibiting drug need not have absolute selectivity.

Figure 4 shows proposed substrates for PTP1B in the phosphorylation cascade that is initiated by the insulin receptor. In this cascade, the insulin receptor itself and IRS1 have been implicated as substrates [7,12]. Another phosphatase, PTEN/MMAC (phosphatase and tensin homolog deleted on chromosome ten/mutated in multiple advanced cancers), is part of this pathway as a phospholipid phosphatase that indirectly stimulates phospho-inositol-3-kinase (PI3K) [13]. Although *in vivo* antisense experiments have validated PTEN as a target in diabetes [14], its identification as a major tumor suppressor – up to half of all human tumors have deleted or mutated PTEN genes [15–18] – disqualifies this phosphatase as a drug target. Nevertheless, this example confirms in a powerful way that blocking phosphatases can stimulate kinase cascades.

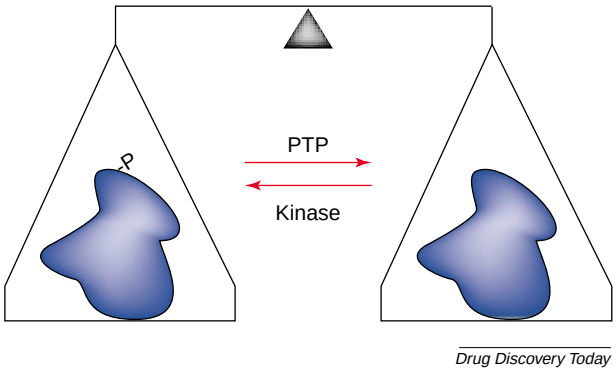


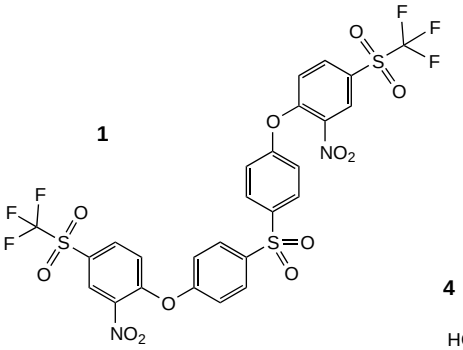
Figure 2. Schematic representation of how protein tyrosine phosphatases (PTPs) and kinases dynamically modify the balance of phosphorylation.

Other PTPs with drug target potential

Here, we will provide a brief, updated [4,5,19] overview of PTPs that are potential drug targets for various disease areas

Figure 3. Companies involved in protein tyrosine phosphatase-1B (PTP1B) inhibitor discovery and representative compounds. Sugen (http://www.sugen.com), ISIS Pharmaceuticals (http://www.isip.com), Aventis (http://www.aventis.com), Ontogen (http://www.ontogen.com), Japan Tobacco (http://www.japantobacco.co.jp), Takeda (http://www.takeda.co.jp), Molecumetics (http://www.molecumetics.com), Pharmacia (http://www.pharmacia.com), Wyeth (http://www.wyeth.com), Novo Nordisk (http://www.novonordisk.com), Abbott (http://www.abbott.com), Korea Research Institute of Chemical Technology (http://www.krict.re.kr/eng/), Albert Einstein College of Medicine (http://www.aecom.yu.edu), Merck-Frosst (http://www.merckfrosst.com).

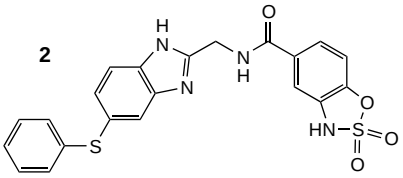
1



Sugen
Trifluoromethyl sulfonyl derivatives
IC_{50} = 400 nM

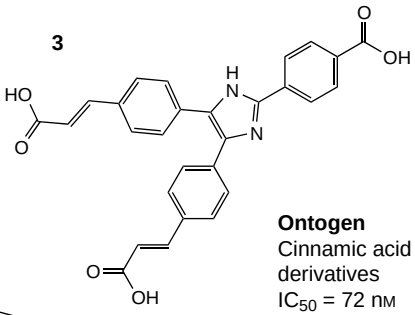
ISIS
ISIS-113715 [350267-87-7]
Antisense oligonucleotide

2



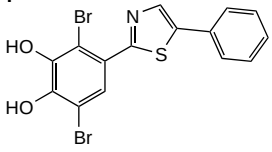
Aventis
Benzooxathiazole derivatives

3



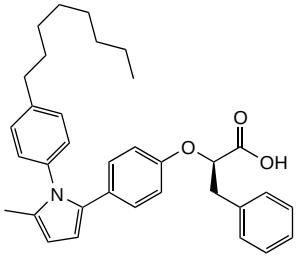
Ontogen
Cinnamic acid derivatives
IC_{50} = 72 nM

4



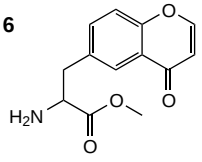
Japan Tobacco
Hydroxyphenyl azole derivatives
IC_{50} = 540 nM

5



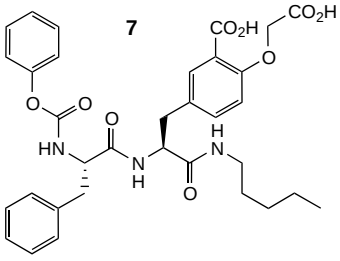
Takeda
Pyrrol phenoxy propionic acid derivatives
IC_{50} = 90 nM

6



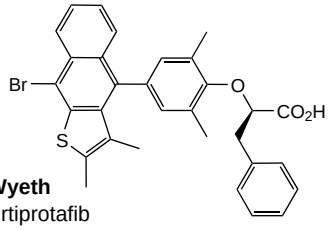
Molecumetics
Phenylalanine derivatives

7



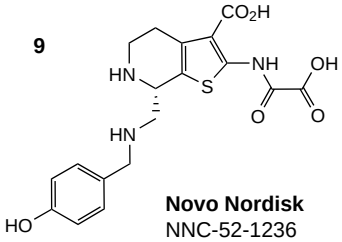
Pharmacia
3'-carboxy-4'-(*O*-carboxymethyl)-tyrosine derivatives
*K*i = 870 nM

8



Wyeth
Ertiprotafib
(Phase II discontinued)
IC_{50} = 384 nM

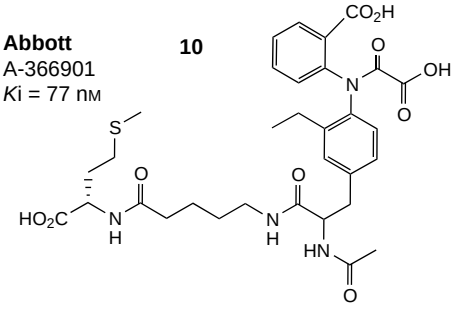
9



Novo Nordisk
NNC-52-1236
*K*i = 200 nM

Abbott
A-366901
*K*i = 77 nM

10



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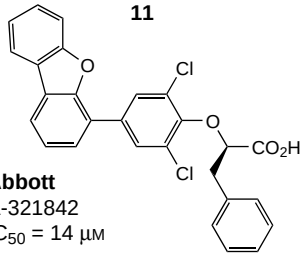
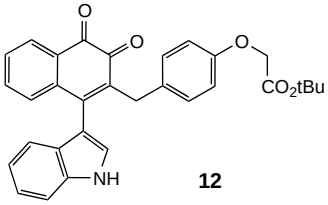


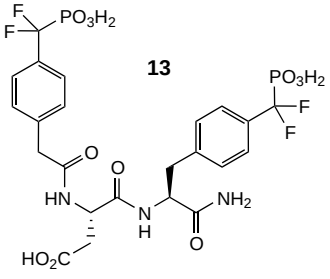
Abbott
A-321842
IC_{50} = 14 μM



12

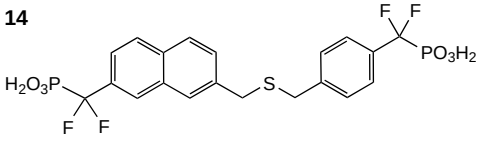
Korea Research Institute of Chemical Technology
1,2-naphtoquinone derivatives
IC_{50} = 650 nM

13



Albert Einstein College of Medicine
4'-phosphonyldifluoromethyl-phenylanaline derivatives
*K*i = 2.4 nM

14



Merck-Frosst
Aryldifluoromethylphosphonic acid derivatives
> 90% inhibition at 10μM

Drug Discovery Today

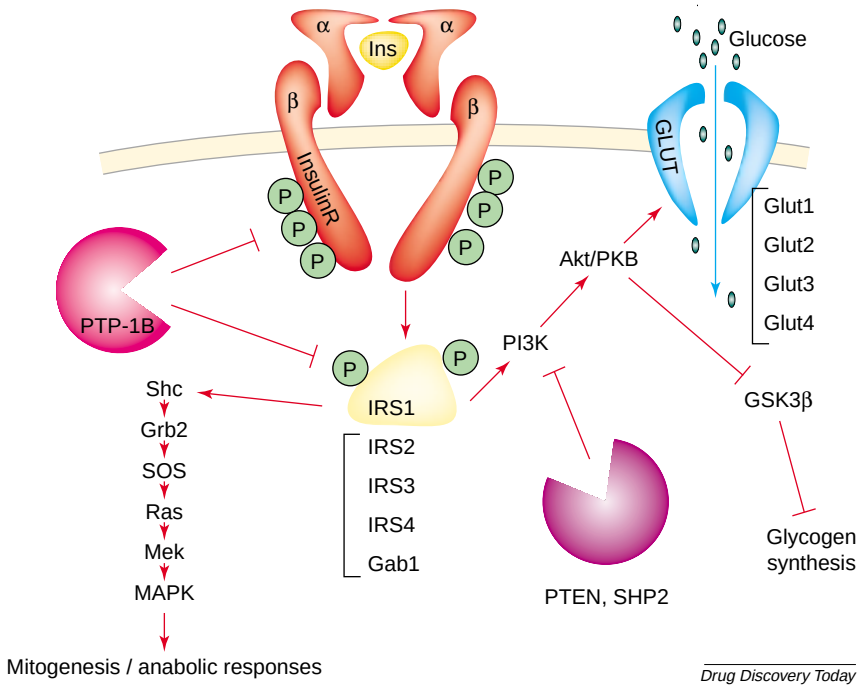


Figure 4. Signaling through the insulin receptor and negative regulation by three phosphatases. Binding of insulin (yellow) to the α-chain of the insulin receptor results in dimerization and receptor β-chain kinase activation. This results in autophosphorylation and phosphorylation of insulin receptor substrate-1 (IRS-1), -2, -3 or -4. The pathway branches into the Ras-MAPK cascade and a phospho-inositol-3-kinase (PI3K) catalyzed pathway that results in the transcriptional and post-translational activation of GLUTs (glucose transporters). Inhibition of three phosphatases, namely PTP1B, PTEN and SHP2, has been shown to stimulate this pathway. Inhibition of PI3K by PTEN and SHP2 is indirect. Abbreviations: Grb-2, growth factor receptor bound protein 2; GSK3β, glycogen synthase kinase 3β; MAPK, mitogen activated protein kinase; MEK, MAP/ERK kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homolog deleted on chromosome ten; SHC, Src-homology-2-containing transforming protein; SHP, Src homology 2-containing tyrosine phosphatase; SOS, son of sevenless protein homolog 1.

(see Table 1 for a summary). Such an assessment is inherently subjective because much of the evidence is still circumstantial.

PTPs involved in immunity and infectious diseases

One of the first PTPs to be identified as such, CD45 [1], is specifically expressed in lymphocytes. Although the precise functions CD45 are still being debated [20–22], it is clear from (again) the mouse KO phenotype that it is involved in lymphocyte T-cell receptor and B-cell immunoglobulin receptor signaling [23]. Thus, blocking CD45 could be therapeutically useful in autoimmune and inflammatory disorders, and graft rejection. However, no CD45-specific inhibitors have yet entered the clinic, possibly because the phosphatase is rather highly expressed.

Two other interesting PTPs that affect the immune system are the two Src-Homology-2 (SH2) -domain containing PTPs SHP1 (SH-2 domain containing PTP-1) and SHP2, which are highly conserved in evolution [24]. There are multiple lines of circumstantial evidence that suggest these PTPs could be therapeutic targets in infectious diseases. An established drug for the treatment of leishmaniasis, sodium stibogluconate, was recently found to strongly inhibit SHP1 and, to a lesser extent, SHP2 [25]. Another pathogen, *Helicobacter pylori*, is known to transduce a protein called CagA into the gastric epidermal cells on which it thrives [26]; recently it was found that CagA, upon phosphorylation, activates SHP2 [27].

Other intracellular parasites bring along their own PTPs. *Vaccinia* virus encodes PTP Yop-H, which is essential for virulence *in vivo* [28,29]. *Mycobacterium tuberculosis*, another intracellular parasite, was found to secrete two active PTPs [30]. Another example is *Salmonella*, which is known to transduce a PTP called SptP into its host cells [31]. For each of these PTPs, multiple intracellular substrates and processes have been proposed, and it is not clear whether some or all of these PTPs target a common pathway. It is likely that these PTPs are aimed at interfering with host signaling, because deleting their genes only affects propagation in the host, and the bacteria involved (*Mycobacteria*, *Shigella*, *Salmonella*) lack kinase cascades themselves. It has been suggested that bacteria have acquired the PTP genes from their eukaryotic hosts through lateral gene transfer [28].

Another potential target in inflammation is PTP-β [or vascular-endothelial-PTP (VE-PTP) for the murine ortholog], which is tissue-specific for endothelial cells. It has recently been shown that this phosphatase specifically dephosphorylates the Tie-2 (Tunica internal endothelial cell kinase-2) receptor kinase [32], which responds to angiopoietin-1. Signaling through this receptor antagonizes blood-vessel leakage [33]. Therefore, PTP-β could be an effective drug target in inflammation as an inhibitor of neutrophil and macrophage extravasation.

Genetic ablation of the PTP-ε gene results in macrophage abnormalities, notably in response to lipopolysaccharide (LPS) [34]. This would suggest that PTP-ε is a potential target in septic shock. The role of PTPs in the immune system was recently reviewed [22,35–39].

Table 1. Overview of PTPs that are potential therapeutic targets, grouped by disease area

Disease area	PTP	Refs
Autoimmune diseases	CD45	[23]
Infectious diseases	SHP1, SHP2	[25,27]
	MPTPA and -B, SptP	[30,31]
Inflammation	PTP-β (VE-PTP), PTP-ε	[32]
Obesity and diabetes	PTP1B	[6–9]
Cancer	PTP-α, PTP-ε, Sap1, GLEPP1, PTP1B	[34,40,46, 68–71]
	Prl-3, Cdc25	[43,44,72,73]
	PTP-ζ (agonists)	[49]
	JSP-1	[51]
Osteoporosis	GLEPP-1 (PTP-oc), PTP-ε	[40,41]
Neurodegeneration	PTP1B, PTP-σ	[52,53]

Abbreviations: GLEPP, glomerular epithelial protein-1 precursor; JSP, Jnk stimulatory phosphatase; MPTPA, *Mycobacterium* PTP-A ; PTP, protein tyrosine phosphatase; SHP, Src homology 2-containing tyrosine phosphatase.

Osteoporosis

Experiments with antisense-mediated inhibition in rabbit osteoclasts have implicated PTP-oc (the rabbit ortholog of glomerular epithelial protein-1 precursor; GLEPP1) as a positive regulator of osteoclastic resorption [40]. These results suggest that blocking GLEPP1 could have a useful anabolic effect in osteoporosis. PTP-ε has also been suggested as a target in osteoporosis, following the discovery that the osteoporosis drug alendronate is, in fact, a PTP-ε-inhibitor [41].

Cancer

Several PTPs (e.g. PTP-α, PTP-ε, Sap1, GLEPP1, PTP1B) have been postulated to dephosphorylate and activate proto-oncogene Src-family kinases. Among these, PTP-α is the best candidate, because mice mutated for this PTP show reduced Src and Fyn kinase activities [42]. Thus, blocking PTP-α could be beneficial in certain types of cancer. Prl-3 is specifically amplified and overexpressed when primary tumors metastasize [43], and is a new and exciting target. Cdc25s are a family of three PTPs that activate cyclin-dependent kinases [44]. Cdc25B is overexpressed in a high proportion (>30%) of primary breast tumors and is currently being explored by several companies as a drug target: inhibitors are effective in tumor models [45].

Predicting whether a PTP is a good drug target for cancer is particularly difficult, and might depend on the type of cancer. For instance, PTP1B has been postulated as an anti-tumor target because it can dephosphorylate and activate c-Src [46], and is overexpressed in ovarian [47] and breast [46] carcinomas. However, its expression is reduced in esophagal cancers [48].

PTPs as drug targets have appeal because they are enzymes that are susceptible to inhibition by small molecules (<500 Da). But some PTPs can also be regulated by their extracellular domains. Many type I 'receptor' PTPs are known to dimerize [49,50]. For some of these, such as PTP-α and CD45, it has been shown that this dimerization results in the repression of their intracellular catalytic domains. The only receptor-PTP for which a ligand has been discovered, PTP-ζ, falls into the latter category [49]. Binding of pleiotrophin to PTP-ζ results in receptor dimerization, inactivation of the enzyme and increased β-catenin phosphorylation and cell proliferation. This model is consistent with both pleiotrophin and β-catenin being associated with mitogenesis and cancer. If correct, a monovalent antibody or pleiotrophin antagonist that keeps PTP-ζ activated might be useful in β-catenin-dependent tumors.

Finally, it was recently discovered that dual-specificity phosphatase JSP1 (Jnk stimulatory phosphatase-1) activates Jun kinase [51]. Because Jun kinase is already well known as a drug target for cancer and a variety of other diseases, JSP1 could also be a target.

Neurodegenerative diseases

Two phosphatases are potentially useful targets in neurodegenerative diseases. PTP-σ KO mice show enhanced neuroregeneration following sciatic nerve injury, as measured by histology, electrophysiology and neuromuscular testing [52]. However, the new neurons also showed directional errors.

Another study showed that, in PTP1B-deficient cells, insulin-growth-like factor-1 (IGF-1) signaling is enhanced [53]. This is not too surprising because the insulin- and IGF-1 receptor autophosphorylation domains are nearly identical, and both are probably good PTP1B substrates. One of the observed effects of IGF-1 hypersensitivity was a protection from apoptosis, which suggests PTP1B inhibitors could have use in neurodegenerative disease with a strong apoptotic component.

Prospects

Lessons from the past for PTP target validation

Since the 19th century, vanadate has been known to have a therapeutic benefit in diabetes [54]. In 1988, the first PTP was identified [1], and by that time the links between vanadate, inhibition of PTPs and insulin signaling were well understood [55,56]. Remarkably, PTP1B was one of the first PTPs to be discovered in 1989 [57], yet it would take 10 more years until its crucial involvement in diabetes was recognized, and this was only because of the PTP1B KO animals' striking phenotype. In the meantime, it had been shown for over half a dozen different PTPs that their overexpression inhibits insulin receptor-initiated signaling

(reviewed in [4]). This illustrates that overexpression of wild-type or dominant negative PTPs compromises both natural PTP substrate-specificity and regulation. Much more so than for other signaling targets, the predictive power of such experiments for PTP target potential is limited.

(No) new PTP targets from the human genome

It had been predicted (and is still believed by some) that the availability of the human genome sequence would greatly expand the quality and diversity of small-molecule drugs, which currently target only ~500 gene products. However, surveys made before [58] and after [59] the release of the human genome sequence draft did not yield vastly different numbers of PTPs, and we have certainly not become any wiser with respect to their functions. A similar conclusion was reached for another drug target class, nuclear receptors [60]. A sobering view on the sudden downwards estimate of the number of genes in the post-genomic world is that it amounts to 'the failure of reduction in biology' [61].

Strategies to PTP target discovery

Few people would recommend reverting to random *in vivo* drug screening; however, the zeal to control and rationally plan and understand every step of the drug discovery process, and the awe of the human genome, might have swung the pendulum too far in the opposite direction. Particularly for targets like PTPs, we must seek shortcuts to find targets more efficiently, even if this comes at the expense of a full understanding of a target's biological function. At present, it is not clear whether the most relevant substrate for PTP1B is the insulin receptor, IRS1, the leptin receptor or Jak2. But it is far from obvious that such knowledge is absolutely required: the unpredicted adverse effects of a drug are at least as likely to be caused by its limited selectivity (i.e. it hitting unforeseen targets) as by unexpected functions of its intended target.

Unfortunately, there is little economic gain from acquiring intellectual property for a drug target (the rewards go to the compounds instead), so most pharmaceutical companies choose to depend on academic results for target validation. But from a purely scientific point of view, validating a target without fully understanding its mechanism is unsatisfactory: academia and industry do not share the same tolerance for shortcuts. This catch-22 situation might well be responsible for the dearth of new drugs that currently enter the market, despite soaring development costs.

Any other good PTP targets out there?

A different question is whether well-characterized PTP targets other than PTP1B, CD45 and Cdc25B have sufficient functional selectivity to enhance specific pathways. The many experiments where antisense reagents enhance specific signaling pathways, and their considerable gene family size, would suggest that many PTPs are selective. The other encouraging observation in this respect is that existing drugs (e.g. alendronate, stibogluconate) are in fact PTP inhibitors. Pinpointing the targets of these drugs further could result in the development of more efficient drugs.

The many PTPs whose molecular biology predicts they might be good drug targets urgently need testing in disease models. The recent development (explosion) of efficient siRNA-based antisense technologies [62–67] is ideally suited to help answer these questions. Similar approaches are being offered commercially by Deltagen (http://www.deltagen.com), Lexicon Genetics (http://www.lexgen.com), Atugen (http://www.atugen.com), ISIS Pharmaceuticals (http://www.isip.com) and others.

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