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Protein-Tyrosine Phosphatase 1B Substrates and Metabolic Regulation

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Abstract

Metabolic homeostasis requires integration of complex signaling networks which, when deregulated, contribute to metabolic syndrome and related disorders. Protein-tyrosine phosphatase 1B (PTP1B) has emerged as a key regulator of signaling networks that are implicated in metabolic diseases such as obesity and type 2 diabetes. In this review, we examine mechanisms that regulate PTP1B-substrate interaction, enzymatic activity and experimental approaches to identify PTP1B substrates. We then highlight findings that implicate PTP1B in metabolic regulation. In particular, insulin and leptin signaling are discussed as well as recently identified PTP1B substrates that are involved in endoplasmic reticulum stress response, cell-cell communication, energy balance and vesicle trafficking. In summary, PTP1B exhibits exquisite substrate specificity and is an outstanding pharmaceutical target for obesity and type 2 diabetes.

Keywords

PTP1B; substrate; obesity; diabetes; ER stress; pyruvate kinase; Eph kinase

1. Introduction

Tyrosine phosphorylation is a key post-translational mechanism that regulates a plethora of cellular processes and is required for maintaining homeostasis. Aberrant changes in tyrosine phosphorylation are often associated with disease states such as metabolic disorders, cancer and cardiovascular disease. Tyrosine phosphorylation is tightly controlled by the dynamic and opposing actions of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs) [1, 2]. PTPs are a superfamily of receptor-like and non-transmembrane proteins, whose members are highly specific, tightly regulated and important modulators of cellular

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signal initiation and termination [2-4]. Classical PTPs contain a 240-250 amino acid catalytic domain specific for phosphotyrosine (pTyr) residues, with a central “signature motif” containing an essential cysteine residue [5]. This review will focus on the prototypical non-receptor protein-tyrosine phosphatase 1B (PTP1B), which has become the subject of intense study and pharmaceutical interest initially owing to its role in metabolic diseases such as obesity and type 2 diabetes. We examine PTP1B cellular location, regulation of enzymatic activity and experimental approaches to identify its substrates. We then highlight the significant findings that implicate PTP1B in metabolic regulation. In particular, insulin and leptin signaling will be discussed as well as PTP1B substrates that are involved in endoplasmic reticulum (ER) stress response, cell-cell communication, energy balance and vesicle trafficking.

2. PTP1B: cellular location and post-translational regulation

PTP1B is an abundant, widely expressed non-receptor phosphatase that was originally purified from human placenta 25 years ago [6]. PTP1B is encoded by the *PTPN1* gene [7] that produces a 435 amino acid protein with a hydrophobic C-terminal sequence that targets PTP1B to the ER [8, 9]. Similar to other members of the PTP family, PTP1B contains the conserved sequence [I/V]HCXXGXXR[S/T] (X is any amino acid) that contains the essential catalytic cysteine [3]. Due to the unique chemical environment of the phosphatase active site, the catalytic cysteine that is located at the base of the active site cleft, has an unusually low acid dissociation constant (pKa) (~5.4). The low pKa enhances the catalytic function of the cysteine as a nucleophile but renders it susceptible to oxidation [5, 10-12], a modification that will be discussed later. Since PTP1B regulates many signaling pathways its function is tightly controlled to avoid aberrant cellular signaling. PTP1B sub-cellular location and post-translational modifications are key modulators of its function.

PTP1B is localized on the cytoplasmic face of the ER by means of a hydrophobic (35 residues) C-terminal sequence [8, 9] that imposes topological constraint on PTP1B ability to access substrates [13]. Despite its sub-cellular location, PTP1B can access substrates during (a) endocytosis, (b) biosynthesis and (c) by the ER network movement in close proximity to the plasma membrane (PM) at apparently specialized regions. (a) Genetic and biochemical studies establish that PTP1B dephosphorylates receptor PTKs (RTKs) including the activated epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and insulin receptor (IR) [14-21]. These findings raised a question: how can the ER-resident PTP1B dephosphorylate RTKs? Fluorescence resonance energy transfer (FRET) studies demonstrate that most of the activated EGFR and PDGFR interact with PTP1B after their endocytosis at specific sites on the surface of the ER [15]. Subsequent studies extended these findings to the IR [17, 21, 22]. In addition, PTP1B is a potential regulator of RTKs endocytosis. PTP1B-EGFR interaction occurs through membrane contact between multivesicular bodies (MVB) and the ER, with PTP1B promoting the sequestration of EGFR to MVB [23]. In line with these findings, the endosomal protein STAM2 which regulates sorting of activated RTKs is a PTP1B substrate [24]. Therefore, PTP1B regulates RTKs dephosphorylation and endocytosis that are two key modulators of RTKs signaling. (b) PTP1B is ideally placed to prevent tyrosine phosphorylation of RTKs during their biosynthesis in the ER. FRET and bioluminescence resonance energy transfer (BRET)

studies demonstrate basal interaction between PTP1B and IR, which can be enhanced by tunicamycin (an inhibitor of RTKs cell surface maturation) [17]. (c) PTP1B also can access PM substrates at regions of cell-cell contact [25-29]. Why can PTP1B dephosphorylate some substrates (such as activated RTKs) after endocytosis, whereas others are targeted on the PM? Quantitative imaging and modeling of protein mobility demonstrate that the ER network comes in close proximity to the PM at apparently specialized sites of cell-cell contact, enabling PTP1B to engage substrates at these regions [30]. Collectively, these studies establish that PTP1B location limits its ability to access substrates but is still capable of interacting with numerous substrates at diverse sub-cellular locations.

Several post-translational modifications regulate PTP1B function including: (a) oxidation, (b) nitrosylation, (c) sulphydration, (d) sumoylation, (e) phosphorylation and (f) proteolytic cleavage. We will briefly highlight some key findings and recent advances (extensively reviewed in [31, 32]). (a) PTP1B, similar to other classical PTPs subfamily members, is susceptible to oxidation by reactive oxygen species (ROS) due to the chemical environment of its catalytic cleft [5, 12]. Oxidation of the active site cysteine abrogates its nucleophilic properties and inhibits PTP1B activity. Oxidation of PTP1B catalytic cysteine rapidly converts the sulphenic acid (S-OH) to a cyclic sulphenamide and is accompanied by significant changes in the architecture of the active site [33, 34]. These structural changes are reversible under physiological conditions and can help protect the enzyme from higher order (S-O₂H and S-O₃H) irreversible oxidation and facilitate reduction back to its active form. Of note, these conformational changes were utilized to generate conformation-sensing intracellular antibodies that maintain PTP1B in an inactive state, and may have therapeutic applications [35]. ROS functions as an intracellular second messenger and activation of RTKs leads to transient production of H₂O₂ which is needed for receptor activation [36]. Stimulus-induced ROS production transiently inactivates PTP1B, which usually exerts inhibitory effects on the system, to initiate a response to the stimulus. Indeed, epidermal growth factor [37] and insulin [38] stimulation leads to reversible oxidation of PTP1B and attenuation of its enzymatic activity. (b) Similar to ROS, reactive nitrogen species inactivate PTP1B [39]. In particular, S-nitrosylation prevents PTP1B active site cysteine from subsequent oxidation when subjected to oxidative stress, and thus plays a protective role against irreversible oxidation [40]. (c) In addition, PTP1B is reversibly inactivated by endogenously generated hydrogen sulphide during the ER stress response through sulphydration of the active site cysteine [41]. Sulphydration of PTP1B provides a potential mechanism for regulating ER stress response. (d) PTP1B is sumoylated on two lysine residues (Lys335 and 347) and its ER-targeting domain is required for maximum sumoylation [42]. Importantly, insulin-induced sumoylation of PTP1B transiently attenuates its enzymatic activity and inhibits the negative effect of PTP1B on insulin signaling [42]. Further, this modification also affects PTP1B-mediated dephosphorylation of emerin, an inner nuclear membrane protein that regulates nuclear architecture [43]. (e) PTP1B is phosphorylated on tyrosine and serine residues, which can either enhance or attenuate its enzymatic activity. Insulin stimulates PTP1B tyrosine phosphorylation (Tyr66, 152 and 153) [44], and results in increased phosphatase activity [45]. On the other hand, insulin induces PTP1B tyrosine phosphorylation and decreases its activity in skeletal muscle and adipose tissue in mice [46]. In addition, PTP1B is phosphorylated on multiple serine residues during

mitosis [47] and stress [48]. Moreover, phosphorylation of PTP1B (Ser50) by Akt attenuates PTP1B enzymatic activity and ability to dephosphorylate the IR [49], possibly as a positive feedback mechanism to potentiate insulin signaling. (f) In activated platelets, calpain-mediated cleavage frees PTP1B from the ER and generates an activated, soluble enzyme in the cytoplasmic space [50]. The reversibly oxidized PTP1B is more vulnerable to calpain proteolysis [51], illustrating how different post-translational modifications may work in tandem to regulate PTP1B activation.

PTP1B sub-cellular location and tight control of enzymatic activity by diverse post-translational modifications illustrate the dynamic regulation of this enzyme and its ability to modulate numerous signaling pathways, likely in a cell/tissue- and stimulus-dependent manner, with high specificity and precision.

3. Identification of PTP1B substrates

PTP1B-substrate interaction is regulated by its non-catalytic motifs and its catalytic domain. PTP1B contains proline-rich motifs that mediate its association with several Src-homology 3 (SH3) domain-containing substrates such as p130^{Cas} and Src [52, 53]. In addition, PTP1B catalytic domain while closely related to other PTPs has intrinsic specificity and preference for select substrates. Crystallographic studies define the molecular basis of PTP1B interaction with the IR and establish that the sequence E/D-pY-pY-R/K is needed for optimal substrate recognition [54]. Indeed, PTP1B exhibits higher affinity (~70 fold) for tandem pTyr-containing peptides compared with mono-pTyr derivatives [54]. Remarkably, this motif can be utilized to predict PTP1B substrates as demonstrated with Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) [55]. Although the tandem pTyr-tyrosine sequence is important for optimal substrate recognition it is not a requirement, as several well-established PTP1B substrates do not harbor it.

Identification of PTP1B substrates is instrumental for understanding its physiological functions. Wild type PTP1B has a catalytic constant (k_{cat}) of $\sim 2 \times 10^3$ molecules/min [56] rendering its association with substrates transient and hard to detect. However, structural studies helped identify PTP1B residues that are important for substrate recognition and catalysis. This enabled researchers to develop the substrate-trapping mutant that retains substrate binding but is catalytically impaired and forms stable complexes with substrates allowing their identification [57]. PTP1B substrate-trap entails mutating the invariant catalytic acid (Asp181) that is conserved in all members of the PTP family [57]. The initial trapping mutant was subsequently improved upon by combining it with another mutation (such as D181A-Q262A) [58-60]. This approach proved powerful in identifying numerous PTP1B substrates and revealed that PTP family members display remarkable substrate specificity in a cellular context [57, 61-66].

4. PTP1B substrates and metabolic regulation

Metabolic syndrome and type 2 diabetes are complex disorders that are associated with sedentary life style, obesity and genetic predisposition [67, 68]. The increased prevalence of these diseases highlights the urgent need to elucidate the underlying molecular mechanisms to aid in therapeutic intervention. PTP1B is an established metabolic regulator in mammals

and a pharmacological target for obesity and type 2 diabetes. We will examine key findings that implicate PTP1B in insulin and leptin signaling, ER stress response, cell-cell communication as well as energy balance and vesicle trafficking (PTP1B substrates that are implicated in metabolism, cancer and immune function are extensively reviewed in [32, 69, 70]).

4.1. Insulin signaling

Insulin is secreted from pancreatic (β -cells and acts as a major regulator of glucose homeostasis through a complex and integrated signaling network [71]. Insulin binding induces IR autophosphorylation on tyrosines (Tyr1158, 1162 and 1163) leading to the recruitment of insulin receptor substrates (IRSs) (Figure 1). These are phosphorylated by the IR and can activate several downstream pathways including the phosphatidylinositol 3-kinase (PI3K)-Akt and MAPK pathways [72, 73]. Numerous studies illustrate the tissue-specific effects of insulin and establish that impaired insulin signaling is central to the development of the metabolic syndrome [74]. PTP1B is an established regulator of insulin signaling as evidenced by biochemical, genetic and inhibitor studies. Initial evidence in *Xenopus* suggests a role for PTP1B in regulating IR phosphorylation and antagonizing insulin signaling [75, 76]. Subsequent studies utilizing overexpression and neutralizing antibodies provide further support for the role of PTP1B in regulating insulin signaling [77, 78]. In addition, substrate-trapping studies identify direct association between PTP1B and IR [44, 79] and IRS1 [80, 81]. Together, these studies implicate PTP1B in IR signaling however, the most compelling evidence was provided by targeted disruption of *PTPN1* in mice [19]. Whole-body PTP1B knockout (KO) mice exhibit increased systemic insulin sensitivity and enhanced glucose tolerance compared with controls [19]. At the molecular level, PTP1B KO mice display enhanced insulin-induced IR phosphorylation in liver and muscle establishing PTP1B as a regulator of insulin signaling *in vivo*. When fed high fat diet, PTP1B KO mice exhibit resistance to weight gain whereas control mice gain weight and develop insulin resistance [19]. These findings were rapidly confirmed and extended in an independently generated PTP1B KO mouse line [20]. Subsequent studies using tissue-specific expression and deletion approaches provide additional insights into the role of PTP1B in insulin signaling. PTP1B over-expression in muscle impairs insulin-stimulated IR activation and decreases muscle glucose uptake [82]. In addition, adenovirus-mediated re-expression of PTP1B in the liver of knockout mice reduces insulin sensitivity, attenuates IR phosphorylation and reveals preferential dephosphorylation of hepatic IR at Tyr1162/1163 by PTP1B [83]. Moreover, mice with muscle-specific PTP1B deficiency have comparable body weight to control mice but exhibit improved systemic insulin sensitivity that is likely due to increased IR phosphorylation [84]. Similarly, mice with liver-specific PTP1B deficiency exhibit increased insulin sensitivity independent of changes in adiposity [85, 86]. Further, mice with myeloid cell-specific PTP1B deficiency exhibit improved glucose tolerance and protection against lipopolysaccharide-induced hyperinsulinemia [87]. These findings sparked interest in developing PTP1B inhibitors for the treatment of obesity and type 2 diabetes. While this led to the generation of potent inhibitors *in vitro*, they did not progress beyond the pre-clinical stage thus far due to delivery and specificity problems [88-90]. However, PTP1B antisense oligonucleotides (ASO; ISIS 113715) reduced PTP1B in liver and adipose, enhanced insulin signaling in the liver and improved systemic insulin

sensitivity in murine models of obesity [91]. Furthermore, ASO administration in monkeys reduces PTP1B expression in liver and adipose and improves insulin sensitivity [92]. Collectively, these studies establish PTP1B as a physiological regulator of insulin signaling and as an outstanding pharmacological target.

4.2. Leptin signaling

Leptin is an adipocyte-derived hormone that regulates feeding and energy homeostasis [93, 94]. Leptin signals through its receptor LepRb, which is associated with JAK2 [95]. Upon leptin binding to LepRb, JAK2 is activated, autophosphorylates and subsequently phosphorylates tyrosine residues along the intracellular tail of LepRb enabling the recruitment of downstream effectors such as signal transducer and activator of transcription 3 (STAT3) [96] (Figure 1). Phosphorylated STAT3 dimerizes and translocates to the nucleus to regulate transcription of target genes. Physiological evidence implicating PTP1B in attenuating leptin signaling through dephosphorylating JAK2 was provided by knockout studies [97, 98]. Mice with combined leptin and PTP1B deficiency display decreased weight gain, reduced adiposity and increased resting metabolic rates [97, 98]. In addition, specific deletion of PTP1B in neurons [99], LepRb-expressing cells [100] and pro-opiomelanocortin (POMC) neurons leads to reduced body mass and adiposity and increased energy expenditure [99]. Furthermore, hypothalamus-specific deletion suggests that the metabolic effects of central PTP1B-deficiency are due to action within the hypothalamus through a leptin receptor-dependent pathway [101]. Together, these studies highlight PTP1B as a target for improving leptin sensitivity.

4.3. Endoplasmic reticulum stress response

Emerging evidence establishes that ER dysfunction is a significant contributor to metabolic diseases, particularly obesity and type 2 diabetes [102, 103]. The ER plays an essential role in protein folding and accumulation of unfolded proteins within the ER lumen causes ER stress [104]. Cells deploy adaptive mechanisms to counter the deleterious effects of ER stress known as the unfolded protein response (UPR) [104]. UPR utilizes three ER transmembrane sensors: protein kinase-like ER kinase (PERK), inositol requiring enzyme 1 α (IRE1 α) and activating transcription factor 6 (ATF6) [103, 105]. These sensors synergize to attenuate stress and maintain homeostasis through increasing the folding capacity of the ER, translational attenuation, ER biogenesis, and ER-associated protein degradation. For example, PERK phosphorylates eukaryotic translation initiation factor 2 (eIF2 α), a modification that blocks initiation of mRNA translation (Figure 1) [106]. Of note, PERK is a serine/threonine kinase but is also tyrosine phosphorylated [107] and its activity is regulated by phosphorylation at Tyr615 [108]. A link between PTP1B and ER stress was established using PTP1B-null fibroblasts which exhibit resistance to IRE1 α -mediated signaling and attenuation of ER stress-induced apoptosis [109]. Subsequent studies showed that mice with liver-specific PTP1B deficiency are protected against high fat diet-induced ER stress and exhibit attenuation of PERK and eIF2 α phosphorylation [85, 110]. In contrast to these findings, PTP1B deficiency in pancreatic β -cell lines leads to increased chemical-induced PERK/eIF2 α signaling [111]. Similarly, PTP1B deficiency in adipose tissue and adipocytes leads to upregulation of PERK-eIF2 α phosphorylation and sensitizes adipocytes to chemical-induced ER stress [112]. In addition, Krishnan *et al*, demonstrated a direct link

between PTP1B and PERK and modulation of ER stress response [41]. ER stress-induced production of hydrogen sulphide in fibroblasts reversibly inactivates PTP1B by sulfhydration of the active site cysteine (see earlier). Importantly, PTP1B inhibition directly promotes PERK phosphorylation and activity during the response to ER stress. Furthermore, this study identifies PERK as a direct substrate of PTP1B [41]. This finding was rapidly confirmed in adipocytes where direct interaction between PTP1B and PERK was established and PERK Tyr615 identified as a mediator of the association [112]. More recently, studies examining the role of PTP1B in ER stress using cultured myotubes point to a requirement for PTP1B for activating PERK [113]. The reason(s) for the seemingly contradictory findings regarding the role of PTP1B in ER stress remain unclear. The effects of PTP1B on ER stress signaling can be general (indirect regulation of one or more pathway) and/or specific (direct regulation of a key target such as PERK). In addition, PTP1B can conceivably regulate the same pathway in a tissue-specific manner to yield different outcomes. This highlights the need to further elucidate the tissue- and stimulus-dependent regulation of ER stress response by PTP1B and its implications in obesity and type 2 diabetes.

4.4. Cell-cell communication

Cell-cell communication is important for metabolic regulation and maintaining homeostasis. For example, pancreatic β cell-cell communication ensures secretion of low amounts of insulin during starvation and high levels during feeding [114]. Eph RTKs and their PM-bound ephrin ligands exhibit a unique feature of bidirectional signaling and are thus well suited for regulating cell-cell communication (Figure 1) [115]. Eph and ephrins are divided into A and B subclasses, with most EphAs binding to ephrin As and most EphBs binding to ephrin Bs [115]. EphA5 signaling is critical for β cell-cell communication and insulin secretion [116]. Under basal conditions, EphA5 signaling inhibits insulin secretion, but after glucose stimulation, EphA5 tyrosine dephosphorylation inhibits its signaling and favors ephrin A5 signaling and insulin secretion [116]. Indeed, Eph RTK pharmacological inhibition enhances glucose-stimulated insulin secretion (GSIS) from mouse and human pancreatic islets [117]. As indicated earlier, ER-resident PTP1B engages PM substrates at regions of cell-cell contact and can regulate cell-cell communication. PTP1B regulates EphA3 function and trafficking [118] and EphA2 tyrosine phosphorylation specifically at regions of cell-cell contact [119]. In addition, pancreatic (endocrine and exocrine) PTP1B deficiency enhances basal and glucose-stimulated EphA5 tyrosine phosphorylation in islets [66]. Further, substrate trapping identifies EphA5 as a PTP1B substrate in pancreatic β -cell line. Mice with pancreatic PTP1B deficiency display comparable glucose tolerance to controls [66]. However, when subjected to a robust challenge (such as prolonged high fat feeding or aging) pancreatic PTP1B knockout mice exhibit mild glucose intolerance and attenuated GSIS, in contrast to the salutary effects of whole-body PTP1B deletion [19, 20] and its deletion in IRS2 knockout mice [120]. Nevertheless, these findings establish association between PTP1B and EphA5; however its contribution to GSIS remains to be determined. It is worth noting that PTP1B likely regulates cell-cell communication by engaging numerous substrates at regions of cell-cell contact. Indeed, Zonula Occludens (ZO1) and p120 catenin are hyper-phosphorylated in PTP1B-null fibroblasts and are thus

putative PTP1B substrates [62]. Regulation of cell-cell communication by PTP1B and its potential contribution to metabolic regulation warrants additional investigation.

4.5. Energy balance

Energy balance is an important component in metabolic homeostasis and emerging evidence suggests regulation of key modulators by PTP1B. AMP-activated protein kinase (AMPK) is a fuel sensing enzyme complex and a regulator of adipose function [121]. PTP1B-deficient brown adipose tissue [122] and brown adipocytes [81] exhibit increased AMPK activity. In addition, recent studies identify pyruvate kinase M2 (PKM2) as a PTP1B substrate [64]. Pyruvate kinase (PK) is a rate-limiting glycolytic enzyme that catalyzes the generation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP [123] (Figure 1). Four isoforms of pyruvate kinase exist in mammals with the M2 isoform expressed in proliferative adult cells and some differentiated tissues such as lung, adipose and pancreatic islets [124]. In addition, most cancer cells have increased expression of PKM2 that facilitates aerobic glycolysis and provides a growth advantage [125, 126]. Indeed, PKM2 deficiency or pharmacological inhibition elicits anticancer effects [126-128]. Moreover, PKM2 pyruvate kinase activity is regulated by binding to phosphotyrosine or becoming phosphorylated under growth stimulation [125, 129]. Of note, PKM2 Tyr105 phosphorylation suppresses its pyruvate kinase activity [129]. Recently, we identified PKM2 as a novel PTP1B substrate in adipocytes and demonstrated that PTP1B deficiency and pharmacological inhibition lead to increased PKM2 total tyrosine and Tyr105 phosphorylation in adipocytes and *in vivo* [64]. Moreover, PKM2 Tyr105 is a key residue that mediates PKM2 interaction with PTP1B. Further, decreased PKM2 Tyr105 phosphorylation in adipose tissue correlates with glucose intolerance and insulin resistance in rodents, non-human primates and humans [64]. These findings establish PKM2 as an exciting PTP1B substrate and highlight the need to investigate the interplay between PTP1B and PKM2 and its potential contribution to the normal metabolic state and to cancer metabolism.

4.6. Vesicle trafficking

Insulin-stimulated glucose uptake in peripheral tissues is mediated by translocation of glucose transporter GLUT4-containing vesicles to the cell periphery and fusion with the PM leading to externalization of GLUT4 [130]. Fusion of vesicles with the PM is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which is tightly regulated to ensure precise trafficking [131]. Munc18 proteins are essential regulators of SNARE-mediated vesicle budding and fusion, and mammalian cells express three isoforms (a, b and c) [132]. Munc18c is solely utilized by adipocytes to regulate insulin-stimulated GLUT4 vesicle exocytosis [133]. In addition, Munc18c tyrosine phosphorylation regulates its interactions and function, and the IR phosphorylates Munc18c (Tyr521) thereby linking insulin signaling to SNARE exocytosis [134] (Figure 1). Munc18c was recently identified as a PTP1B substrate and PTP1B deficiency in adipocytes and adipose tissue leads to increased Munc18c tyrosine phosphorylation [65]. In addition, Munc18c Tyr218/219 and Tyr521 mediate its interaction with PTP1B. Importantly, biochemical and functional studies establish that insulin-induced Munc18c tyrosine phosphorylation in general, and Tyr218/219 and Tyr521 in particular, regulate its

associations and facilitate SNARE-mediated delivery of GLUT4 to the PM [65]. Collectively, these studies identify PTP1B as a regulator of Munc18c phosphorylation and function in adipocytes. Additional studies that decipher PTP1B regulation of Munc18 isoforms in insulin-responsive tissues will likely yield new insights into GLUT4 trafficking and may provide modalities to increase glucose transport.

5. Concluding remarks

The alarming increase in metabolic syndrome highlights the urgent need for effective therapeutic interventions. As this review illustrates, PTP1B is an outstanding pharmacological target for obesity and type 2 diabetes. Numerous PTP1B substrates have been identified with some serving as key components in metabolic signaling including insulin, leptin, ER stress, cell-cell communication, energy balance and vesicle trafficking, and it is likely that additional metabolic substrates will be identified. Deciphering PTP1B-substrates interactions and their biological significance will yield valuable insights into metabolic regulation. While PTP1B inhibition is actively pursued for therapeutic intervention and novel approaches will likely aid in developing small molecule inhibitors for clinical evaluation, targeting PTP1B substrates may be easier and should be considered as a complementary approach to combat obesity and type 2 diabetes.

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Abbreviations

PTKs	protein-tyrosine kinases
PTPs	protein-tyrosine phosphatases
pTyr	phosphotyrosine
PTP1B	protein-tyrosine phosphatase 1B
ER	endoplasmic reticulum
EGFR	epidermal growth factor receptor
PDGFR	platelet-derived growth factor receptor
IR	insulin receptor

FRET	fluorescence resonance energy transfer
MVB	multivesicular bodies
BRET	bioluminescence resonance energy transfer
ROS	reactive oxygen species
SH3	Src-homology 3
JAK2	Janus kinase 2
TYK2	tyrosine kinase 2
IRS	insulin receptor substrate
PI3K	phosphatidylinositol 3-kinase
KO	knockout
LepRb	leptin receptor
STAT3	signal transducer and activator of transcription 3
POMC	pro-opiomelanocortin
UPR	unfolded protein response
PERK	protein kinase-like ER kinase
IRE1α	inositol requiring enzyme 1 α
ATF6	activating transcription factor 6
eIF2α	eukaryotic translation initiation factor 2
GSIS	glucose-stimulated insulin secretion
ZO1	Zonula Occludens
AMPK	AMP-activated protein kinase
PKM2	pyruvate kinase M2
PEP	phosphoenolpyruvate
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor

Figure 1. Schematic representation of PTP1B-substrates interaction and metabolic regulation
Insulin signaling and vesicle trafficking: Insulin activates the IR leading to IRS1 phosphorylation and subsequent activation of downstream signaling pathways. PTP1B attenuates insulin signaling by dephosphorylating the endocytosed IR and IRS1. GLUT4-containing vesicles (GSV) interact with several molecules as they are delivered towards the cell periphery then are tethered and fused to the plasma membrane. In adipocytes, the IR phosphorylates Munc18c while PTP1B dephosphorylates it thereby linking insulin signaling directly to SNARE exocytosis. Leptin signaling: Leptin binds to the LepRb and leads to JAK2 activation and subsequent phosphorylation of LepRb tyrosine residues enabling STAT3 recruitment. Phosphorylated STAT3 translocates to the nucleus to regulate transcription. PTP1B attenuates leptin signaling by dephosphorylating JAK2. Cell-cell communication: The ER network comes in close proximity to the plasma membrane at specialized regions of cell-cell contact (right corner), enabling ER-resident PTP1B to engage substrates (such as EphA5) at these sites. Energy balance: PKM2 is a rate-limiting glycolytic enzyme that catalyzes the generation of pyruvate and ATP from PEP and ADP. PKM2 is a PTP1B substrate in adipocytes, and PTP1B deficiency increases PKM2 overall and site-specific tyrosine phosphorylation. ER stress response: Under basal conditions PERK is maintained inactive by its association with the molecular chaperone BiP. When improperly folded proteins accumulate in the ER, BiP dissociates from PERK which is activated. PERK phosphorylates eIF2 α , a modification that blocks initiation of translation. PTP1B directly

interacts with and dephosphorylates PERK. Tyrosine phosphorylated residues are indicated by green ovals and serine phosphorylated residue is indicated by green oval with white stripes.