Protein Tyrosine Phosphatase-1B Negatively Regulates Insulin Signaling in L6 Myocytes and Fao Hepatoma Cells.

Running Title: Overexpression of PTP1B in L6 and Fao cells.

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ABSTRACT

Insulin signaling is regulated by tyrosine phosphorylation of the signaling molecules, such as the insulin receptor and insulin receptor substrates (IRSs). Therefore, the balance between protein tyrosine kinases and protein tyrosine phosphatase activities is thought to be important in the modulation of insulin signaling in insulin-resistant states. We thus employed the adenovirus-mediated gene transfer technique and analyzed the effect of overexpression of a wild-type protein tyrosine phosphatase-1B (PTP1B) on insulin signaling in both L6 myocytes and Fao cells. In both cells, PTP1B overexpression blocked insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1 by more than 70%, and resulted in a significant inhibition of the association between IRS-1 and the p85 subunit of phosphatidylinositol 3-kinase and Akt phosphorylation as well as MAP kinase phosphorylation. Moreover, insulin-stimulated glycogen synthesis was also inhibited by PTP1B overexpression in both cells. These effects were specific for insulin signaling, because platelet derived growth factor (PDGF)-stimulated PDGF receptor tyrosine phosphorylation and Akt phosphorylation were not inhibited by PTP1B overexpression. The present findings demonstrate that PTP1B negatively regulates insulin signaling in L6 and Fao cells, suggesting that PTP1B plays an important role in insulin resistance in muscle and liver.
INTRODUCTION

After insulin binds to its own receptor, the insulin receptor is phosphorylated on its tyrosine residues, and tyrosine kinase activity is activated. The activated insulin receptor binds to insulin receptor substrates (IRSs) via the YMXM motif, and IRSs are also phosphorylated on tyrosine residues (1). The tyrosine phosphorylated IRSs activate their downstream signaling molecules, such as phosphatidylinositol (PI) 3-kinase and p21ras. Since tyrosine phosphorylation is essential for insulin signaling, the balance of activities between protein tyrosine kinases and protein tyrosine phosphatases (PTPase) appears to be very important for insulin’s effects.

Several lines of evidence demonstrate that PTPase activity is increased in insulin resistant states such as obesity and type 2 diabetes mellitus. It has been reported that obese human subjects have increased PTPase activity in skeletal muscle and adipose tissue (2, 3), and a 10% body weight reduction causes a decrease in overall adipose tissue PTPase activity with enhanced insulin sensitivity (4). Furthermore, insulin infusion in vivo produces a rapid 25% suppression of soluble-PTPase activity in muscles of insulin-sensitive subjects, but this response is severely impaired in subjects who are insulin resistant (5). Moreover, increased PTPase activity is also observed in the liver (6) and skeletal muscle (7) of diabetic rats. Taken together, abnormalities of PTPase activity are thought to be important to understand the molecular mechanism of insulin resistance.

Previous studies showed that the tandem-domain transmembrane enzymes, leukocyte antigen related (LAR) and leukocyte common antigen-related phosphatase (LRP)/RPTP-α, and the intracellular, single domain enzymes, protein tyrosine phosphatase-1B (PTP1B) and SHP2 are candidate PTPases for the regulation of the insulin signaling pathway. In particular, PTP1B directly interacts with the activated insulin receptor (8), and exhibits the highest specific activity towards IRS-1 (9). We have also reported that exposing Rat 1 fibroblasts expressing human insulin receptors...
to a high glucose condition impairs insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1 due to the increased PTP1B expression and activity (10). Furthermore, overexpression of PTP1B by the electroporation method reduces the level of GLUT4 on the cell surface in primary cultured rat adipose cells (11). Moreover, it is reported that mice lacking the PTP1B gene show increased insulin sensitivity and resistance to high-fat diet induced obesity, which is supported by enhanced insulin-induced tyrosine phosphorylation of the insulin receptor and IRS-1 in muscle and liver (12). Thus, PTP1B appears to play an important role in the regulation of insulin signaling.

In the present study, we employed the adenovirus mediating gene transfer technique, and analyzed the effect of PTP1B overexpression on insulin signaling in model cells of insulin target tissues, such as L6 myocytes and Fao cells. In both cells, PTP1B overexpression markedly inhibited insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1, and resulted in a significant inhibition of the association between IRS-1 and the p85 subunit of PI 3-kinase and Akt phosphorylation as well as MAP kinase phosphorylation. Moreover, glycogen synthesis was also inhibited under both basal and insulin-stimulated conditions. The present findings demonstrate that PTP1B negatively regulates insulin signaling in muscle and liver cells.
EXPERIMENTAL PROCEDURES

Materials. Human insulin was provided by Eli Lilly Inc. (Indianapolis, IL). Anti-PTP1B antibody, anti-IRS-1 antibody, and anti-p85 N-SH2 antibody were purchased from Upstate Biotechnology Incorporated (Lake Placid, NY). Anti-phospho-Akt antibody and anti-phospho-MAP kinase antibody were from New England Biolabs (Beverly, MA). Horseradish peroxidase conjugated phosphotyrosine antibody (RC20H) and insulin receptor antibody were from Transduction Laboratories (Lexington, KY). Horseradish peroxidase-linked anti-rabbit and anti-mouse antibodies, anti-ERK2 antibody, anti-Akt1 antibody, and anti-PDGF receptor antibody were from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Dulbecco’s modified Eagles (DME) medium and fetal calf serum (FCS) were obtained from Life Technologies. All radioisotopes were obtained from Du-Pont-NEN (Boston, MA). XAR-5 film was obtained from Eastman-Kodak (Rochester, NY). All other reagents and chemicals were purchased from Sigma (St. Louis, MO).

Cell Culture. L6 cells, which were provided by Dr. A. Klip (The Hospital for Sick Children, Toronto, Canada), were grown and maintained in MEM-α containing 50 U/ml penicillin, 50 µg/ml streptomycin and 10% FCS in a 5% CO₂ environment. The cells were reseeded in the appropriate culture dishes and, after reaching subconfluency, the medium was changed to MEM-α containing 2% FCS. The medium was then changed every two days until the cells were fully differentiated, typically after 5 days. Fao cells, which were from Dr. C. R. Kahn (Joslin Diabetes Center, Boston, MA), were grown and maintained in DME medium containing 50 U/ml penicillin, 50 µg/ml streptomycin and 10% FCS in a 5% CO₂ environment. Prior to experimentation, the cells were trypsinized and reseeded in the appropriate culture dishes. The Ad-E1A-transformed human embryonic kidney cell line 293 was cultured in DME high glucose medium.
medium containing 50 U/ml penicillin, 50 µg/ml streptomycin and 10 % FCS in a 5% CO₂ environment.

**Preparation of Recombinant Adenovirus.** The recombinant adenovirus containing PTP-1B cDNA was generated as previously described (13). PTP-1B wild-type cDNA was subcloned into pACCMVPpASR(+) plasmid (14). This plasmid contains 1.3 map units of adenovirus 5 (Ad5) left end, cytomegalovirus early promoter, PUC19 polylinker site, and SV40 poly-A signal sequences, followed by map units 9-18 of the Ad5 genome. The resulting recombinant plasmid was then cotransfected into 293 packaging cells with pJM17 plasmid (15), which carries Ad5 genomic DNA and propagated as previously described (16). Mature recombinant Ad5 encoding PTP-1B wild-type was thus generated after in vivo homologous recombination between these two plasmids. Since 293 cells were originally derived from adenovirus transformation, the missing E1 gene function of pJM17 was provided in trans. The resulting recombinant virus containing the PTP1B was denoted as Ad5-PTP1B, and was replication defective (at least in cells lacking the E1 region of the adenovirus), but fully infectious.

**Cell Treatment.** L6 myocytes and Fao cells were infected at a multiplicity of infection (MOI) of 10-50 plaque formation units (pfu)/ cell for 1 or 2 h with stocks of either a control recombinant adenovirus (Ad5-ctrl) containing the cytomegalovirus promoter, pUC 18 polylinker, a fragment of the SV40 genome or the recombinant adenovirus containing PTP1B (Ad5-PTP1B). Transduced cells were incubated for 56 h at 37°C in 5% CO₂ and appropriate medium with 2% heat inactivated serum, followed by incubation in the starvation media required for the assay. The efficiency of adenovirus mediated gene transfer was approximately 90% as measured by immunocytochemistry.
Western Blotting. Ad5-ctrl or Ad5-PTP1B infected cells were starved for 16 h in DME regular glucose medium with 0.05% FCS. The cells were stimulated with 100 ng/ml of insulin for 5-10 min at 37°C and lysed in a solubilizing buffer containing 20 mM Tris, 1 mM EDTA, 140 mM NaCl, 1% Nonidet P-40 (NP-40), 50 U/ml aprotinin, 1 mM Na3VO4, 1 mM PMSF, 50 mM NaF, pH 7.5 for 30 min at 4°C. The cell lysates were centrifuged to remove insoluble materials. For Western blot analysis, whole cell lysates (20 µg protein per lane) were denatured by boiling in Laemmli sample buffer containing 100 mM dithiothreitol and resolved by SDS-PAGE. Gels were transferred to nitrocellulose by electroblotting in Towbin buffer containing 20% methanol. For immunoblotting, membranes were blocked and probed with specified antibodies. Blots were then incubated with horseradish peroxidase-linked second antibody followed by chemiluminescence detection, according to the manufacturer’s instructions (Amersham Pharmacia Biotech).

Glycogen Synthase Activity. Glycogen synthase activity was determined as described previously (17). Differentiated L6 myocytes were infected with Ad5-PTP1B or Ad5-ctrl at 50 MOI for 1 h, and grown in medium containing heat inactivated serum (2%) for 72 h. The cells were serum and glucose starved in DME no glucose, 0.1% BSA, 2 mM pyruvate medium for 3 h, then stimulated with or without 200 ng/ml of insulin for 30 min in 5 mM glucose containing medium. Cells were washed with ice-cold PBS three times, scraped in the buffer containing 50 mM Tris-HCl, 10 mM EDTA, 100 mM KF (pH 7.4), and sonicated. After centrifugation, the protein concentration was measured. 10 µg protein was used to determine the ability to stimulate incorporation of [14C]UDP-glucose into glycogen in the presence or absence of glucose 6-phosphate.
**Glycogen Synthesis.** Glycogen synthesis was measured as described previously (18) with some modification. Fao cells were infected with Ad5-PTP1B or Ad5-ctrl at 10 MOI for 2 h, and grown in medium containing 2% heat inactivated serum for 56 h. The cells were serum starved for 16 h and the medium was then replaced with DME medium containing 1% BSA. The cells were incubated with [\(^{14}\text{C}\)]glucose (0.4 µCi/well) and 100 ng/ml insulin for 2 h in a CO\(_2\) incubator, washed with PBS 3 times, and lysed with 2N NaOH at 55°C. The synthesized [\(^{14}\text{C}\)]glycogen was precipitated with cold glycogen in 66% ethanol, washed, and the radioactivity was measured.

**Statistics.** The values are expressed as mean ± SEM, unless otherwise stated. Scheffe’s multiple comparison test was used to determine the significance of any differences among more than three groups. P<0.05 was considered significant.
RESULTS

Expression of PTP1Bwt in L6 Myocytes and Fao Cells. Differentiated L6 myocytes and Fao cells were infected with recombinant adenovirus expressing wild-type PTP1B as described in experimental procedures. Following 72 h incubation, the cells were lysed, and analyzed by SDS-PAGE followed by Western blotting with anti-PTP1B antibody (Fig. 1). Small amounts of endogenous PTP1B were observed in both cell lines. PTP1B was expressed in a dose-dependent manner in each cell line. Total phosphatase activity also increased dose-dependently in PTP1B overexpressing cells as reported previously\(^1\) (13) (data not shown).

Effects of PTP1B Expression on Tyrosine Phosphorylation of Insulin Receptor and IRS-1, and Association between IRS-1 and the p85 Subunit of PI 3-Kinase. The infected cells were stimulated with 100 ng/ml of insulin for 5 min and lysed. The cell lysates (200-500 \(\mu\)g protein) were immunoprecipitated with anti-insulin receptor antibody or anti-IRS-1 antibody. The immunocomplexes were analyzed by Western blotting with anti-phosphotyrosine antibody. Tyrosine phosphorylation of the insulin receptor and IRS-1 were decreased more than 70% in the Ad5-PTP1B infected cells in both cell lines (Fig. 2A, C, F, G, I, L). The membranes were stripped and reblotted with anti-p85 antibody. The association between IRS-1 and the p85 subunit of PI 3-kinase also decreased in PTP1B overexpressing cells (Fig. 2D, J) and paralleled the reduction of IRS-1 tyrosine phosphorylation (Fig. 2C, I).

Effect of PTP1B Expression on Akt Phosphorylation. Next, we studied the downstream signaling of PI 3-kinase. Either PTP1B expressing L6 myocytes or Fao cells were stimulated with insulin for 10 min, lysed, and analyzed by Western blotting with phospho-specific-Akt antibody (Fig. 3). Overexpression of PTP1B almost completely inhibited insulin-induced Akt phosphorylation in both cell lines.
Phosphorylation of p70S6 kinase, which is another downstream molecule of PI3-kinase, was also decreased by PTP1B overexpression (data not shown).

**Effect of PTP1B Expression on Glycogen Synthesis.** Differentiated L6 myocytes were infected with PTP1B and control adenoviruses at 50 MOI for 1 h. Following 72 h incubation, the cells were serum- and glucose-starved for 3 h, and stimulated with 200 ng/ml of insulin for 30 min. Overexpression of PTP1B almost completely inhibited insulin-stimulated glycogen synthase activity (control 2.51±0.29% vs. PTP1B 1.55±0.19%, p<0.02, Fig. 4).

Next, we examined [¹⁴C]glucose incorporation into glycogen in Fao cells. The adenovirus infected Fao cells were incubated with insulin and [¹⁴C]glucose for 2 h, and glycogen synthesis was measured as described in experimental procedures. As shown in Figure 5, overexpression of PTP1B inhibited glycogen synthesis at both basal (control 126.4±0.99% vs. PTP1B 88.5±7.24%, p<0.01) and insulin-stimulated conditions (control 190.9±6.93 vs. PTP1B 121.9±7.80, p<0.001).

**Effect of PTP1B Expression on MAP Kinase Phosphorylation.** The PTP1B expressing cells were stimulated with insulin for 10 min, lysed, and subjected to SDS-PAGE followed by Western blotting with anti-phospho-specific MAP kinase antibody (Fig. 6). PTP1B expression totally inhibited insulin-stimulated MAP kinase phosphorylation in both cell lines.

**PTP1B Overexpression did not Affect PDGF-Stimulated Receptor and Akt Phosphorylation.** Finally, we examined the effects of PTP1B on platelet derived growth factor (PDGF) signaling. Either Ad5-ctrl or Ad5-PTP1B infected L6 myocytes were stimulated with 30 ng/ml of PDGF for 5 min, lysed, and then analyzed by SDS-PAGE followed by Western blotting with anti-phosphotyrosine antibody or
anti-phospho-specific Akt antibody (Fig. 7). PTP1B expression did not affect either
PDGF-induced PDGF receptor tyrosine phosphorylation or Akt phosphorylation.
Thus, the observation concerning PTP1B overexpression appears to be specific for
insulin signaling.
DISCUSSION

We previously reported that exposing Rat 1 fibroblasts expressing human insulin receptors to high glucose conditions impaired the insulin-stimulated tyrosine phosphorylation of the insulin receptor and IRS-1 due to the increased expression and activity of PTP-1B (10). More direct evidence has shown that overexpression of PTP1B reduced the level of GLUT4 on the cell surface in primary cultured rat adipose cells (11). Recently, it was reported that the PTP1B knockout mice showed increased insulin sensitivity and resistance to high-fat diet induced obesity with enhanced insulin-induced tyrosine phosphorylation of the insulin receptor and IRS-1 in muscle and liver (12). However, in those mice, insulin-induced tyrosine phosphorylation of the insulin receptor and IRS-1 in adipose tissue was not affected. Another study also showed that increased insulin sensitivity in PTP1B-deficient mice was tissue specific, as insulin-stimulated glucose uptake was elevated in skeletal muscle, whereas adipose tissue was unaffected (19). Furthermore, it has been reported that overexpression of PTP1B decreased insulin-induced tyrosine phosphorylation of the insulin receptor and IRS-1 by about 50%, but did not affect the glucose transport in 3T3-L1 adipocytes (20). Therefore, the role of PTP1B for insulin effects appears to be tissue specific.

In the current studies, we overexpressed PTP1B wild-type in L6 myocytes and Fao cells using adenovirus mediated gene transfer to analyze the effect of PTP1B on insulin signaling. PTP1B overexpression markedly inhibited insulin-stimulated MAP kinase phosphorylation, Akt phosphorylation, and glycogen synthesis in both L6 myocytes and Fao cells with marked inhibition of tyrosine phosphorylation of the insulin receptor and IRS-1. These inhibitory effects were specific for insulin signaling, because PTP1B overexpression did not affect PDGF-stimulated phosphorylation of PDGF receptor and Akt (Fig. 7).

Similar to data derived from animal studies, the inhibitory effects of PTP1B on insulin signaling appears cell-type specific. In 3T3-L1 adipocytes, PTP1B
overexpression inhibited tyrosine phosphorylation of the insulin receptor and IRS-1 by about 50%, but Akt phosphorylation and glucose uptake were intact (20). The inhibition of tyrosine phosphorylation of the insulin receptor and IRS-1 were greater in L6 myocytes and Fao cells, so this may be one explanation for the different effects of PTP1B among these cells. It has been reported that PTP1B anchors to the endoplasmic reticulum, and is activated after release into the cytosol by truncation of its COOH-terminus (21, 22). Localization of overexpressed PTP1B may be different in each cell line. Another explanation for these findings may be the different expression levels of endogenous PTP1B. In 3T3-L1 adipocytes, PTP1B may not play an important role because its expression level is relatively low, and other phosphatases may be important. Thus, overexpression of PTP1B might show smaller effects than in L6 and Fao cells. In L6 myocytes and Fao cells, both insulin-stimulated MAP kinase phosphorylation and PI 3-kinase pathway were inhibited by PTP1B overexpression. However, only insulin-stimulated MAP kinase phosphorylation was significantly blocked by PTP1B overexpression in 3T3-L1 adipocytes (20). This difference may be able to explain the cell-type specificity of PTP1B effects.

Adenovirus-mediated liver-specific PTP1B overexpression in rats did not cause insulin resistance in the recent study by Wang et al. The reason for this difference between in vivo and in vitro study is unclear. One possible explanation is the difference of the expression level. PTP1B overexpression was increased by about 2 to 3 fold in the liver of the rats in this study, whereas it was increased by 20 to 30 times in our cells.

PTP1B overexpression showed different effects among L6 myocytes, Fao cells, and 3T3-L1 adipocytes. PTP1B caused insulin resistance in muscle and liver cells, but had small effects in adipocytes (20) in the culture system, similar to the observation of the PTP1B knockout mice (12, 19). It was reported that insulin signaling was impaired in muscle, but was not in liver of IRS-1 knockout mice by rescuing up-
regulated IRS-2 expression (23), but the disruption to the IRS-2 gene in mice caused severe insulin resistance in the liver (24). Furthermore, it was reported that IRS-3 plays an important role in adipose tissue of IRS-1 knockout mice (25). These findings demonstrate that specific molecules mediate insulin signaling in different tissues. RPTPα or another phosphatase might be important in adipose tissue.

In conclusion, the present findings indicate that PTP1B negatively regulates insulin signaling in muscle and liver cells. To investigate the activity of PTP-1B may be important to understand the molecular mechanism of insulin resistance in the type 2 diabetic patients.
REFERENCES


15
**FOOTNOTES**

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**Abbreviations**  2DOG; 2-deoxy-glucose, IRS; insulin receptor substrate, MAP kinase; mitogen-activated protein kinase, PMSF; phenylmethylsulfonyl fluoride, PTPase; protein tyrosine phosphatase, SDS-PAGE; sodium dodecyl sulfate polyacrylamide gel electrophoresis, PI 3-kinase; phosphatidylinositol 3'-kinase, PDGF; platelet derived growth factor

FIGURE LEGENDS

Fig. 1  Expression of PTP1B in L6 Myocytes and Fao Cells. The differentiated L6 myocytes (A) and Fao cells (B) were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at the indicated MOIs for 1 hour (h) or 2 h, respectively. After 72 h, the cells were lysed, and analyzed by SDS-PAGE followed by Western blotting with anti-PTP1B antibody. Each Western blot is a representative of three independent experiments.

Fig. 2  Effects of PTP1B Overexpression on Tyrosine Phosphorylation of Insulin Receptor and IRS-1, and Insulin-Induced Association of IRS-1 and the p85 Subunit of PI 3-Kinase. The differentiated L6 myocytes (A-F) and Fao cells (G-L) were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 50 MOI or 10 MOI for 1 hour (h) or 2 h, respectively. After 56 h, the cells were starved for 16 h, and stimulated without or with 100 ng/ml of insulin for 5 minutes (min). Then, the cells were lysed and immunoprecipitated with anti-insulin receptor antibody (A, B, G, H) or anti-IRS-1 antibody (C, D, E, I, J, K). Immunocomplexes were analyzed by Western blotting with phosphotyrosine antibody (RC20H) (A, C, G, H), anti-insulin receptor antibody (B, H), anti-p85 antibody (D, J), and anti-IRS-1 antibody (E, K). Each Western blot is a representative of four independent experiments. Tyrosine phosphorylation level was quantitated by NIH Image, and the percentage of phosphorylation (count of tyrosine phosphorylation / protein amount) was calculated. The graph shows the means ± SEM of the percentage of uninfected and insulin-stimulated cells (F, L). * indicates the difference from insulin-stimulated values in the cells with control virus at p<0.01.

Fig. 3  Effect of PTP1B Overexpression on Akt Phosphorylation in L6 Myocytes and Fao Cells. The differentiated L6 myocytes (A, B) and Fao cells (C, D) were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 50 MOI or 10 MOI for 1 hour
(h) or 2 h, respectively. Following infection, the cells were serum starved (16 h), incubated in the absence or presence of insulin (100 ng/ml) for 10 min. Total cell lysates (20 µg) were subjected to SDS-PAGE and immunoblotted with phospho-specific Akt antibody (A, C). The membrane was stripped and re-blotted with anti-Akt1 antibody (B, D). The Western blot is a representative of four independent experiments.

**Fig. 4** Effect of PTP1B Overexpression on Glycogen Synthase Activity in L6 Myocytes. The differentiated L6 myocytes were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 50 MOI for 1 h. After 72 h, the cells were serum and glucose starved in DME no glucose, 0.1% BSA, 2 mM pyruvate medium for 3 h, then stimulated with or without 200 ng/ml insulin for 30 min in medium containing 5 mM glucose. Results are expressed as means ± SEM of percentage of glycogen synthase index (% GSI) for three independent experiments. % GSI was determined as (activity without G-6-P / activity with G-6-P) x 100. * indicates the difference from insulin-stimulated values in the cells with control virus at p<0.02.

**Fig. 5** Effect of PTP1B Overexpression on Glycogen Synthesis in Fao Cells. Fao cells were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 10 MOI for 2 h. After 56 h, the cells were serum starved for 16 h, then the medium was changed to DME with 1% BSA. The cells were incubated with 100 ng/ml of insulin and [14C]glucose for 2 h in 5% CO2 incubator. [14C]glucose incorporation into glycogen are expressed as % of the basal value of non-transfected cells. The graph shows the means ± SEM of three experiments. * indicates the difference from basal values in the cells with control virus at p<0.01. ** indicates the difference from insulin-stimulated values in the cells with control virus at p<0.001.
Fig. 6 Effect of PTP1B Overexpression on MAP Kinase Phosphorylation in L6 Myocytes and Fao Cells. The differentiated L6 myocytes (A, B) and Fao cells (C, D) were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 50 MOI or 10 MOI for 1 hour (h) or 2 h, respectively. Following infection, cells were serum starved (16 h), incubated in the absence or presence of insulin (100 ng/ml) for 10 min. Total cell lysates (20 µg) were subjected to SDS-PAGE and immunoblotted with phospho-specific MAP kinase antibody (A, C). The membrane was stripped and re-blotted with anti-Erk2 antibody (B, D). Each Western blot is a representative of four independent experiments.

Fig. 7 Effect of PTP1B Overexpression on PDGF-Stimulated Receptor and Akt Phosphorylation in L6 Myocytes. The differentiated L6 myocytes were infected with Ad5-ctrl (ctrl) or Ad5-PTP1B (PTP1B) at 50 MOI for 1 hour (h). Following infection, the cells were serum starved (16 h), incubated in the absence or presence of PDGF (30 ng/ml) for 5 minutes. Total cell lysates (20 µg) were subjected to SDS-PAGE and immunoblotted with phosphotyrosine antibody (RC20H) (A) or phospho-specific Akt antibody (C). The membrane was stripped and re-blotted with anti-PDGF receptor antibody (B) or anti-Akt1 antibody (D). The Western blot is a representative of three independent experiments.
Figure 1

(A) PTP1B -

(B) PTP1B -

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Figure 2

(A) 1 2 3 4 5 6
IR -
(B) IR -
(C) IRS-1 -
(D) p85 -
(E) IRS-1 -

(F) Phosphorylation level
Insulin Virus - - - - - -
no + + + + + +
 ctrl + + + + + +
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IR
IRS-1

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Figure 2

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Phosphorylation level

** by guest on November 6, 2017 http://www.jbc.org/ Downloaded from
Figure 3

(A) p-Akt

(B) Akt

(C) p-Akt

(D) Akt

Insulin

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Figure 4

![Graph showing % GSI for different conditions.

- Insulin: - (-), + (+)
- Virus: no, ctrl, PTP1B

% GSI values are indicated with error bars, with one star (*) indicating a significant difference.](http://www.jbc.org/)}
**Figure 5**

![Graph showing glucose incorporation into glycogen](image)
Figure 6

(A) p-MAPK

(B) MAPK

(C) p-MAPK

(D) MAPK

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1 2 3 4 5 6
Figure 7