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Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor β to Ras

(Src homology 2/tyrosine kinase/signal transduction)

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ABSTRACT **Protein-tyrosine-phosphatase SHPTP2 (Syp/PTP-1D/PTP2C) is the homologue of the *Drosophila* corkscrew (*csw*) gene product, which transmits positive signals from receptor tyrosine kinases. Likewise, SHPTP2 has been implicated in positive signaling from platelet-derived growth factor receptor β (PDGFR). Upon PDGF stimulation, SHPTP2 binds to the PDGFR and becomes tyrosine-phosphorylated. We have identified tyrosine-542 (pY^{542}TNI) as the major *in vivo* site of SHPTP2 tyrosine phosphorylation. The pY^{542}TNI sequence conforms to the consensus binding site for the SH2 domain of Grb2, which, by association with Sos1, couples some growth factor receptors to Ras. Following PDGF stimulation, Grb2 binds tyrosine-phosphorylated SHPTP2. Moreover, a mutant PDGFR lacking its SHPTP2 binding site displays markedly reduced Grb2 binding. These data indicate that phosphorylation of SHPTP2 couples Grb2 to PDGFR *in vivo*, providing a mechanism for Ras activation by PDGFR and for positive signaling via SHPTP2 and Csw.**

Receptor protein-tyrosine kinases (RPTKs) are critical regulatory enzymes for cell growth, differentiation, and development. Binding of a growth factor to its cognate RPTK promotes receptor dimerization and "trans-phosphorylation" on multiple tyrosine residues (1). These phosphotyrosines serve as docking sites for secondary signaling molecules containing Src homology 2 (SH2) domains, most of which are also RPTK substrates. SH2 domains mediate specific, high-affinity interactions with phosphotyrosine-containing peptides (2).

Two general classes of SH2-containing proteins exist: enzymes, such as GTPase activating protein (GAP) and phospholipase Cγ, and adapters, such as Grb2 and the p85 subunit of phosphatidylinositol 3-kinase (PI-3K), which lack intrinsic enzymatic activity and consist of SH2 and SH3 domains (2). SH2-mediated binding can relocate cytosolic enzymes to their substrates (e.g., GAP to Ras proteins) (3). Alternatively, SH2/RPTK binding may increase the enzymatic activity of SH2-containing proteins either directly, as for PI-3K (4–6), or indirectly, by promoting receptor-directed tyrosine phosphorylation, as for phospholipase C-γ1 (7). Adapters (e.g., PI-3K) can couple non-SH2-containing catalytic subunits to activated RPTKs.

These early events culminate in the activation of downstream targets such as Ras. Recently, the pathway from some RPTKs to Ras has been elucidated. For example, Grb2 binds directly to the activated epidermal growth factor receptor (EGFR) via its SH2 domains and in turn, via its SH3 domains, is bound to the guanine nucleotide-exchange protein Sos1 (8–13). However, other RPTKs that activate Ras, such as platelet-derived growth factor receptor β (PDGFR), have no obvious Grb2 binding site(s) (14).

SHPTP2 (15), also known as Syp (16), PTP-1D (17), and PTP-2C (18), is a ubiquitously expressed protein-tyrosine-phosphatase (PTP) and is the homologue of the *Drosophila csw* gene product, Csw (19). SHPTP2 contains two SH2 domains, a PTP domain, and a C-terminal hydrophilic domain with several potential phosphorylation sites. SHPTP2 binds directly to the activated PDGFR (at phosphotyrosine-1009, pY 1009) via its N-terminal SH2 domain, as well as to the activated EGFR and insulin receptor substrate 1. Moreover, SHPTP2 becomes tyrosine-phosphorylated following PDGF and EGF, but not insulin, stimulation (16, 17, 20, 21).

Several lines of evidence suggest that SHPTP2 and *csw* transduce positive signals from RPTKs (see *Discussion*). A model for SHPTP2 signaling was proposed in which tyrosine phosphorylation of its C terminus creates a binding site for a positive signaling SH2-containing protein (20). We have identified the major site of tyrosine phosphorylation of SHPTP2 *in vivo*. Phosphorylation of this site can be catalyzed by the PDGFR, which creates a binding site (pY^{542}TNI) for Grb2. Thus, tyrosine-phosphorylated SHPTP2 may function as an adapter, linking Grb2 to the activated PDGFR.

MATERIALS AND METHODS

Cell Culture. ATWT and AT1009 cells (22, 23) were maintained in Dulbecco's modified Eagles's medium (DMEM) with 10% fetal bovine serum plus antibiotics. BALB/c 3T3 cells were maintained in DMEM with 10% calf serum plus antibiotics.

Recombinant Protein Expression and Purification. Full-length or C-terminally truncated human SHPTP2 was expressed and purified as described (24). To generate full-length SHPTP2 as a fusion protein, an *Eco*RI fragment containing the SHPTP2 cDNA (15) was ligated into the *Eco*RI site of pGEX-2T (Pharmacia), to encode glutathione *S*-transferase (GST) and 14 nt of the SHPTP2 5′ untranslated region (resulting in linker amino acids PGGRN), followed by the complete sequence of SHPTP2. GST–SHPTP2 ΔC terminus and GST–SHPTP2 C terminus were constructed by use of PCR amplification. For GST–SHPTP2 ΔC terminal cDNA encoding SHPTP2 aa 1–489 was amplified and an *Eco*RI site was introduced at the 3′ end by using the primers 5′-TCACTATAGGGCGAATTGGGTACC-3′ (T7) and 5′-CTCTTCTTGAATTCTGCGCTGTA-3′. For GST–SHPTP2 C terminus, a cDNA fragment encoding aa 491–593 was amplified

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Abbreviations: EGFR, epidermal growth factor receptor; GST, glutathione *S*-transferase; PDGFR, platelet-derived growth factor receptor β; PTP, protein-tyrosine-phosphatase; RPTK, receptor protein-tyrosine kinase; SH2, Src homology 2; TLE, thin-layer electrophoresis.

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and an *Eco*RI site was introduced at the 5′ end, by using the primers 5′-TACAGCGCAGAATTCAAGAAGAG-3′ and 5′-ACGCCAAGCTCGAAATTAACCCTC-3′ (T3). PCR products were digested with *Eco*RI and ligated into pGEX-2T.

Point mutations converting Y542, Y547, or Y580 to phenylalanine (F) were generated by PCR overlap extension (25) of aa 461–593, using the following primer sets: for GST–SH-PTP2-Y542F, 5′-GGGCACGAATTTACAAATAATAAG-3′ and T3 with 5′-CCCGTGCTTAAATGTTTATTATTC-3′ and T7; for GST–SHPTP2-Y547F, 5′-CAAATATTAAGTT-TTCTCTAGCGG-3′ and T3 with 5′-CCGCTAGAGAAAAC-TTAATATTTG-3′ and T7; for GST–SHPTP2-Y580F, 5′-GTCTTTGAAAACGTAGGCCTGATG-3′ and T3 with 5′-CAGAAACTTTTGCATCCGGACTAC-3′ and T7. These fragments were digested with *Pst*I (5′ internal site) and *Eco*RI (3′ internal site), and subjected to a three-way ligation with *Eco*RI/*Pst* I-digested cDNA encoding aa 1–460 of SHPTP2, and *Eco*RI-digested pGEX-2T. The double mutant GST–SHPTP2-Y542/580F was generated from the corresponding single mutants. PCR-generated fragments were confirmed by dideoxy sequencing. GST fusion proteins were expressed and purified as described (26).

***In Vitro* Binding Assays.** BALB/c 3T3 cells were made quiescent by incubation for 48 hr in DMEM with 0.5% calf serum plus antibiotics and stimulated for 15 min with PDGF (50 ng/ml; Oncogene Science). Lysates (20) were incubated with 3–5 μg of glutathione-agarose beads containing GST–Grb2 or GST alone for 1 hr at 4°C. Bound complexes were resolved by SDS/8% PAGE and transferred onto Immobilon (Millipore).

Immunoprecipitation and Immunoblotting. Equal amounts of protein from unstimulated and PDGF-stimulated ATWT, AT1009, or BALB/c 3T3 cells were immunoprecipitated with affinity-purified anti-SHPTP2 583 antibodies (20) at 10 μg/ml or with 10–20 μl of rabbit anti-Grb2 antiserum (Upstate Biotechnology) for 2 hr. Immune complexes were collected on protein A-Sepharose, separated by SDS/PAGE, and transferred onto Immobilon (Millipore).

Immunoblots were probed with monoclonal anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology) at 1 μg/ml, a 1:500 dilution of monoclonal anti-PTP-1D/SHPTP2 (Transduction Laboratories, Lexington, KY), or a 1:500 dilution of monoclonal anti-Grb2 (Transduction Laboratories). Donkey anti-rabbit IgG or sheep anti-mouse IgG peroxidase-linked secondary antibodies (Amersham) were used at 1:10,000. Blots were developed with enhanced chemiluminescence (Amersham).

Tryptic Phosphopeptide Mapping and Phospho Amino Acid Analysis. Fusion proteins were eluted from glutathione beads with 100 mM Hepes, pH 7.4/150 mM NaCl/0.1% Triton X-100/5 mM dithiothreitol/20 mM glutathione. PDGFR immunoprecipitates prepared from ATWT cells were incubated with eluted proteins and [γ-^{32}P]ATP (27). For metabolic labeling experiments, quiescent ATWT cells were preincubated for 1 hr in phosphate-free DMEM plus 0.1% dialyzed fetal bovine serum and labeled for 3 hr with [^{32}P]orthophosphate (1 mCi/ml; NEN; 1 mCi = 37 MBq). PDGF was added for the final 15 min. SHPTP2 was immunoprecipitated with affinity-purified 583 antibodies and bands of interest were excised and subjected to tryptic phosphopeptide mapping and phospho amino acid analysis (28).

RESULTS

PDGFR Phosphorylates SHPTP2 *in Vitro* and *in Vivo* at the Same Site. SHPTP2 was immunoprecipitated from PDGF-stimulated, [^{32}P]orthophosphate-labeled ATWT cells, which express high levels of PDGFR (22, 23). Two-dimensional phosphopeptide mapping of the SHPTP2 band yielded two major fragments, A′ and A″, when thin-layer electrophoresis (TLE) was carried out at pH 1.9 (Fig. 1*a*). Phospho amino acid analysis of peptides A′ (Fig. 1*b*) and A″ (data not shown) revealed almost exclusively phosphotyrosine. The broad appearance of A′ and A″ suggested that they might represent partial digestion or variable phosphorylation of the same peptide; subsequent analysis (see below) supported the former explanation. Several minor phosphopeptides were also obtained. These were more easily resolved by TLE at pH 8.9 (data not shown) but have not been analyzed further.

Recombinant SHPTP2 (non-GST) phosphorylated *in vitro* by PDGFR immunoprecipitates also yielded two tryptic phosphopeptides similar to those generated from immunoprecipitated SHPTP2 (Fig. 1*c*). Indeed, *in vitro* and *in vivo* tryptic phosphopeptides of SHPTP2 comigrated (Fig. 1*d*), suggesting that the same site(s) was phosphorylated *in vivo* and *in vitro*. Analogous data were obtained for peptides from *in vitro* phosphorylated recombinant SHPTP2 resolved at pH 8.9 (data not shown). These data suggested that the PDGFR phosphorylates SHPTP2 *in vivo*, allowing us to use *in vitro* phosphorylated SHPTP2 to map the *in vivo* tyrosine phosphorylation site(s).

PDGFR Phosphorylates SHPTP2 at Tyrosine-542. GST fusion proteins with either deletions or point mutations of full-length SHPTP2 were constructed (Fig. 2). Tryptic phosphopeptides A′ and A″ were also generated from *in vitro* phosphorylated GST–SHPTP2, along with variable amounts of two additional peptides (Fig. 3*a*, dotted lines). GST alone is not a PDGFR substrate (data not shown), so these additional phosphopeptides arise from SHPTP2. Phosphorylation of a fusion protein lacking the SHPTP2 C-terminal 64 aa (GST–SHPTP2 ΔC terminus) resulted in the elimination of phosphopeptides A′ and A″, plus the additional phosphopeptides noted above (dotted lines) (Fig. 3*b*). Similar results were obtained with recombinant truncated SHPTP2 lacking GST (data not shown). Moreover, a GST fusion protein containing only the SHPTP2 C-terminal 64 aa yielded phosphopeptides A′ and A″ and two phosphopeptides (B′ and B″) that comigrated with the peptides indicated by the dotted lines in Fig. 3*a* (Fig. 3 *c* and *d*). Analogous results were observed at pH 8.9 (data not shown). Thus, the major tyrosine phosphorylation site(s) resides within the C-terminal 64 aa of SHPTP2, which contain three tyrosines: Y542, Y547, and Y580. These residues were mutated to phenylalanine within GST–full-length SHPTP2. The Y547F mutation had no effect on the map of *in vitro* phosphorylated SHPTP2 (data not shown). However, the Y542F mutation resulted in the loss of fragments A′ and A″ (Fig. 3*e*), whereas the Y580F mutation

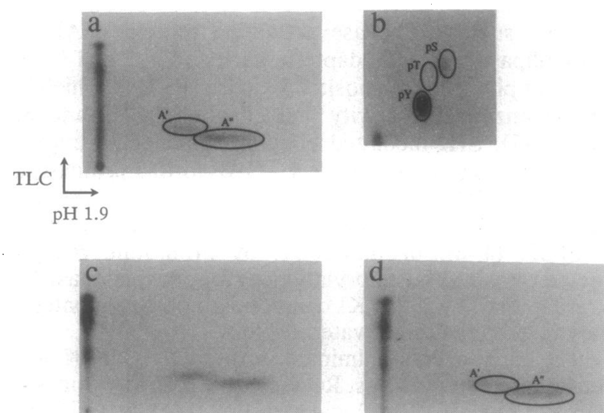

FIG. 1. (*a*) Tryptic phosphopeptide maps TLC followed by TLE at pH 1.9 of *in vivo* labeled SHPTP2 from PDGF-stimulated ATWT cells. A′ and A″ correspond to major phosphopeptides. (*b*) Phospho amino acid analysis of peptide A′. Ovals indicate phospho amino acid standards (pS, phosphoserine; pT, phosphothreonine; pY, phosphotyrosine). (*c*) Tryptic phosphopeptide map of SHPTP2 (without GST) phosphorylated *in vitro* by PDGFR. (*d*) Mix of *in vivo* and *in vitro* phosphotryptic peptides.

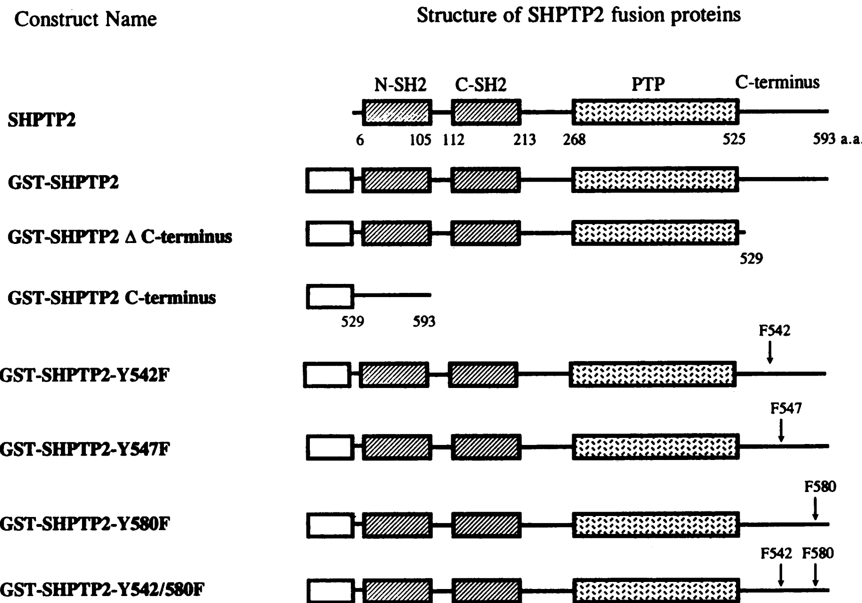

FIG. 2. Schematic representation of recombinant SHPTP2 fusion proteins. The amino acid numbers correspond to the sequence of human SHPTP2 (24). Arrows indicate the positions of tyrosine-to-phenylalanine point mutations.

eliminated peptides B′ and B″ (Fig. 3*g*). The GST–SHPTP2 phosphopeptide map was otherwise unaltered, as shown by mixing experiments (Fig. 3 *f* and *h*). These data suggest that peptides A′ and A″ contain Y542, whereas peptides B′ and B″ contain Y580. Mutations Y542F and Y580F eliminated phosphopeptides A′ and A″ as well as B′ and B″ (data not shown). Since only A′ and A″ are generated from *in vivo* phosphorylated SHPTP2 (Fig. 1*a*), our results establish Y542 of SHPTP2 as the major *in vivo* phosphorylation site. Similar results were obtained with TLE at pH 8.9 (data not shown).

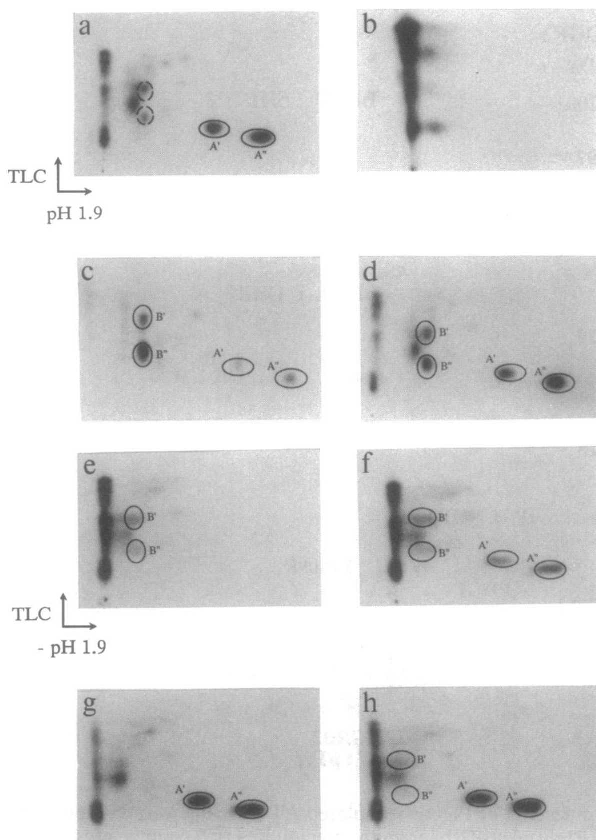

FIG. 3. Tryptic phosphopeptide maps of GST–SHPTP2 fusion proteins phosphorylated *in vitro* by PDGFR. (*a*) GST–SHPTP2. (*b*) GST–SHPTP2 ΔC terminus. (*c*) GST–SHPTP2 C terminus. (*d*) Mix of *a* and *c*. (*e*) GST–SHPTP2-Y542F. (*f*) Mix of *a* and *e*. (*g*) GST–SHPTP2-Y580F. (*h*) Mix of *a* and *g*.

SHPTP2 Directly Mediates Grb2 Association with PDGFR. The sequence surrounding Y542, pY[542]TNI, conforms to the consensus binding site (pYXNX) for the SH2 domain of Grb2 (10, 14, 29, 30). Since Grb2 binding to SHPTP2 might explain Ras activation following PDGF stimulation, we asked whether SHPTP2 could bind Grb2 *in vitro*. GST–Grb2, but not GST alone, bound a 70-kDa protein that was recognized by anti-SHPTP2 and anti-phosphotyrosine antibodies in lysates from PDGF-stimulated BALB/c 3T3 cells (Fig. 4). The SHPTP2/Grb2 interaction is mediated directly via the Grb2 SH2 domain, since the Grb2 SH2 domain alone binds tyrosine-phosphorylated recombinant SHPTP2 (data not shown). Recombinant SHPTP2 (lacking GST) is phosphorylated at a single site, Y542 (Fig. 1*c*), suggesting that SHPTP2 binds Grb2 directly through pY[542]TNI. SHPTP2 also complexes with Grb2 *in vivo*. SHPTP2 was detectable in Grb2 immunoprecipitates, and Grb2 was present in SHPTP2 immunoprecipitates from PDGF-stimulated cells (Fig. 5 *a* and *b*). Gratuitous SH2/phosphotyrosine interactions could occur in ATWT cells, which overexpress the PDGFR. However, Grb2 also coimmunoprecipitated with SHPTP2 in a

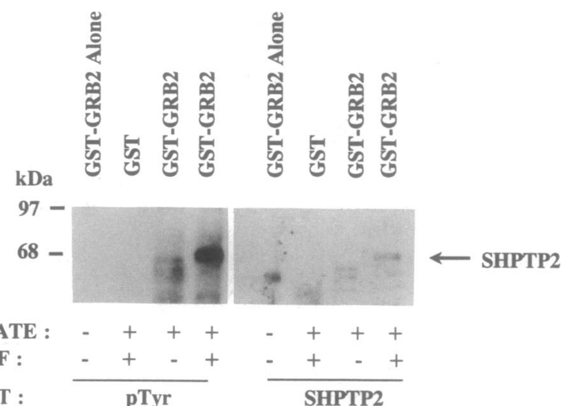

FIG. 4. Tyrosine phosphorylated SHPTP2 binds Grb2 *in vitro*. Lysates from unstimulated (−) or PDGF-stimulated (+) BALB/c 3T3 cells were incubated with GST–Grb2, bound proteins were separated by SDS/8% PAGE, and blots were probed with monoclonal anti-PTP-1D/SHPTP2 or anti-phosphotyrosine (4G10) (pTyr) antibodies. GST–Grb2 was also incubated with buffer alone in the absence of cell lysate.

PDGF-dependent manner from BALB/c 3T3 cells (Fig. 5c). Our SHPTP2 antibodies immunoprecipitate at least 80% of total cellular SHPTP2 (data not shown) allowing us to estimate that ≈5% of total cellular Grb2 is complexed with SHPTP2 (Fig. 5c).

To establish whether SHPTP2 is responsible for recruiting Grb2 to the PDGFR, we utilized a canine epithelial cell line (AT1009) expressing a PDGFR mutant (Y1009F) lacking the SHPTP2 binding site (22, 23). Reimmunoprecipitation experiments established that tyrosine-phosphorylated SHPTP2 binds to the activated PDGFR (data not shown). If SHPTP2 mediates Grb2 association with the PDGFR, immunoprecipitation of Grb2 should result in PDGFR coimmunoprecipitation from ATWT cells but not from AT1009 cells. Indeed, Grb2 immunoprecipitates from AT1009 cells contained substantially reduced amounts of tyrosine-phosphorylated PDGFR (Fig. 5d), suggesting that SHPTP2 mediates the major proportion of Grb2 association with the PDGFR. A trivial explanation for diminished coimmunoprecipitation would be if SHPTP2 were not tyrosine-phosphorylated at Y542 in AT1009 cells; however, SHPTP2 from AT1009 cells generated tryptic phosphopeptides A′ and A″ (data not shown). Thus, the substantial reduction in PDGFR association with Grb2 in AT1009 cells is most likely due to the inability of the mutant receptor to bind SHPTP2.

DISCUSSION

Lechleider et al. (20) suggested three models for SHPTP2 signaling. (i) The N-terminal SH2 domain of SHPTP2 binds to an activated RPTK, leaving the C-terminal SH2 domain free to transmit signals. Although weak binding of the C-terminal SH2 domain to the PDGFR was observed (20), we have not detected C-terminal SH2 binding to other phosphotyrosine-containing proteins (R. J. Lechleider and B.G.N., unpublished data). (ii) The PTP domain sends a positive signal. Following PDGF stimulation, Src-like PTKs associate with and are activated by the PDGFR (31, 32), thus SHPTP2 could dephosphorylate the negative regulatory phosphotyrosine residues of Src-like kinases (33–35). This model is also consistent with enhanced SHPTP2 phosphatase activity upon binding of a phosphotyrosine peptide to its SH2 domains (36). However, ATWT and AT1009 cells display equivalent levels of c-Src activation following PDGF stimulation (A. Kashishian, J. Cooper, R. J. Lechleider, and B.G.N., unpublished data). Although these results do not exclude a role for the PTP domain in positive signaling, Src-like kinases seem unlikely targets. (iii) Tyrosine phosphorylation of the SHPTP2 C terminus creates a binding site for a positive signaling SH2-containing protein. Our data suggest that this model accounts for positive signaling by SHPTP2 downstream of the PDGFR.

We have identified Y542 as the major site of phosphorylation of SHPTP2 in vivo and shown that PDGFR is the likely kinase. Several lines of evidence suggest that pY[542]TNI of SHPTP2 couples Grb2 to the activated PDGFR in vivo: (i) tyrosine-phosphorylated (pY[542]TNI) recombinant SHPTP2 binds GST–Grb2 directly in vitro, (ii) GST–Grb2 binds SHPTP2 in a phosphotyrosine-dependent manner in lysates from PDGF-stimulated cells, (iii) tyrosine phosphorylation of SHPTP2 in vivo occurs at Y542, (iv) Grb2 and SHPTP2 coimmunoprecipitate in a PDGF-dependent manner, (v) SHPTP2 is the only SH2-containing protein known to bind the PDGFR at pY1009, and (vi) substantially lower levels of the PDGFR coimmunoprecipitate with Grb2 from AT1009

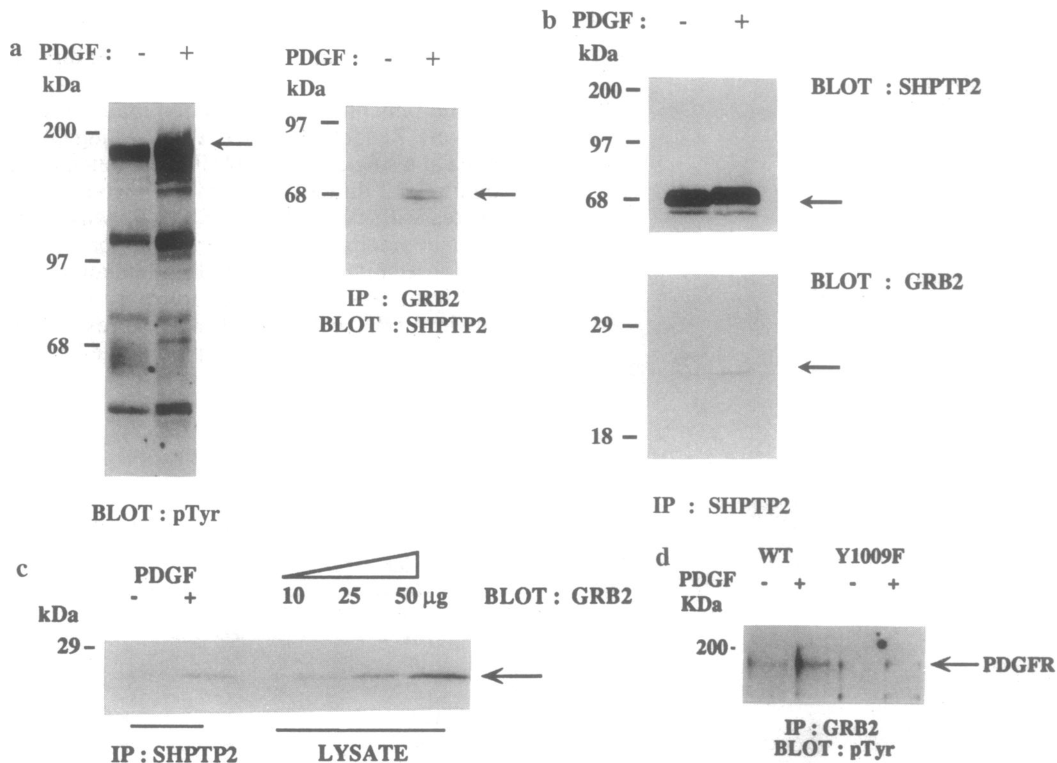

FIG. 5. SHPTP2 complexes with Grb2 *in vivo*. (*a*) (*Left*) Cell lysates from quiescent or PDGF-stimulated ATWT cells were immunoblotted with anti-phosphotyrosine antibodies. Arrow indicates the activated PDGFR. (*Right*) Anti-Grb2 immunoprecipitates (IP) from lysates in *a* were immunoblotted with anti-PTP-1D/SHPTP2 antibodies. Arrow indicates SHPTP2. (*b*) Anti-SHPTP2 immunoprecipitates from lysates in *a* were immunoblotted with anti-Grb2 (*Lower*) or anti-PTP-1D/SHPTP2 (*Upper*) antibodies. Arrows indicate SHPTP2 (*Upper*) or Grb2 (*Lower*). (*c*) Lysates (600 µg) from quiescent or PDGF-stimulated BALB/c 3T3 cells were immunoprecipitated with affinity-purified 583 antibodies. Immune complexes and the indicated amounts of total lysate were immunoblotted with monoclonal anti-Grb2 antibodies. (*d*) ATWT (WT) and AT1009 (Y1009F) cells were stimulated with PDGF (+) or left unstimulated (−). Equal amounts of lysate were immunoprecipitated with anti-Grb2 antibodies and immunoblotted with anti-phosphotyrosine antibodies. Arrow indicates tyrosine phosphorylated PDGFR.

cells than from ATWT cells. Although our data suggest that most Grb2 association with the PDGFR is mediated through SHPTP2, we detect a small amount of Grb2 association in AT1009 cells. Thus, it will be important to verify the biological relevance of the SHPTP2/Grb2 interaction by appropriate mutagenesis studies.

Our data provide an explanation for two observations regarding PDGFR signaling. Valius *et al.* (37) showed that compared with ATWT cells, AT1009 cells require higher concentrations of PDGF for optimal growth, and Valius and Kazlauskas (38) reported that a PDGFR lacking most of its phosphorylation sites but retaining Y1009 was capable of PDGF-induced Ras activation. These studies suggest that SHPTP2 sends signals from the PDGFR by activating Ras. Notably, murine SHPTP2 (16), Csw (19), and *Xenopus* SHPTP2 (R. Freeman and B.G.N., unpublished data) all conserve the Grb2 binding site. Our data predict that Csw sends its positive signals downstream of Torso by binding to the *Drosophila* protein Drk (39, 40), placing Csw upstream of *Drosophila* Ras1. This hypothesis is supported by embryo microinjection studies, in which dominant negative Ras produces a *csw*-like phenotype and *csw* mutants can be rescued by activated Ras (41).

Although *in vivo* SHPTP2 tyrosine phosphorylation was detected only on Y542, Y580 was variably phosphorylated *in vitro*. Y580 could be phosphorylated transiently *in vivo* but rapidly autodephosphorylated, preventing its detection by tryptic phosphopeptide mapping. Indeed, SHPTP2's relative SHPTP1 rapidly autodephosphorylates *in vitro* (27). Although we think it unlikely, we cannot exclude the possibility that canine SHPTP2 is so divergent from human SHPTP2 that (in ATWT cells) it is not a substrate for the human PDGFR at Y580 or that the tryptic phosphopeptide containing Y580 from canine SHPTP2 is not soluble at pH 1.9 or pH 8.9. Conceivably, Y580 could be a substrate for other RPTKs *in vivo*. The sequence around Y580 also conforms to the Grb2 binding site. Phosphorylation of both Y542 and Y580 *in vivo* might transmit a more robust downstream signal. Interestingly, Syp, which appears to be an SHPTP2 splicing variant (16), lacks Y580. Perhaps alternatively spliced forms of SHPTP2 have different signaling capacities.

Unlike previously described SH2-containing proteins, SHPTP2 contains both adapter and intrinsic enzymatic functions. The role of the PTP domain of SHPTP2 in PDGF signaling remains unclear. Binding of a phosphotyrosine peptide ligand activates the PTP *in vitro* (36). Upon RPTK binding, activated SHPTP2 might attenuate the RPTK signal by dephosphorylating the RPTK, RPTK-associated proteins, and/or itself. Alternatively, part of SHPTP2's positive signal may be transmitted via dephosphorylation of as yet unidentified substrates. SHPTP2 is not tyrosine-phosphorylated following insulin stimulation, although it does associate with insulin receptor substrate 1 (21). If SHPTP2 sends a positive signal from the insulin receptor, it presumably must use a different mechanism.

Li *et al.* (42) reported that SHPTP2 forms a complex with Grb2 following PDGF stimulation. Our data confirm and extend these findings by identifying (*i*) the likely site on SHPTP2 for interaction with Grb2 and (*ii*) PDGFR as the probable SHPTP2 kinase. Further studies are required to assess the generality of this model for RPTK signal transduction involving SHPTP2 and Csw.

Note Added in Proof. Recent studies indicate that SHPTP2, when transiently overexpressed in COS-1 cells, is phosphorylated on Y542 and Y580 in response to PDGF and that both of these sites can bind Grb2.

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