SHP-1 Associates with Both Platelet-derived Growth Factor Receptor and the p85 Subunit of Phosphatidylinositol 3-Kinase*

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The Src homology 2 (SH2)-containing protein tyrosine phosphatase 1, SHP-1, is highly expressed in all hematopoietic cells as well as in many non-hematopoietic cells, particularly in some malignant epithelial cell lines. In hematopoietic cells, SHP-1 negatively regulates multiple cytokine receptor pathways. The precise function and the targets of SHP-1 in non-hematopoietic cells, however, are largely unknown. Here we demonstrate that SHP-1 associates with both the tyrosine-phosphorylated platelet-derived growth factor (PDGF) receptor and the p85 subunit of phosphatidylinositol 3-kinase in MCF-7 and TRMP cells. Through the use of mutant PDGF receptors and performing peptide competition for immunoprecipitation, it was determined that SHP-1 independently associates with the PDGF receptor and p85 and that its N-terminal SH2 domain is directly responsible for the interactions. Overexpression of SHP-1 in TRMP cells transfected with the PDGF receptor markedly inhibited PDGF-induced c-fos promoter activation, whereas the expression of three catalytically inactive SHP-1 mutants increased the c-fos promoter activation in response to PDGF stimulation. These results indicate that SHP-1 might negatively regulate PDGF receptor-mediated signaling in these cells. Identification of the association of SHP-1 with the PDGF receptor and p85 in MCF-7 and TRMP cells furthers our understanding of the function of SHP-1 in non-hematopoietic cells.

Protein tyrosine phosphorylation is critical in many cellular processes including signal transductions, neoplastic transformation, and the control of the mitotic cycle. These cellular processes are regulated by the activities of both protein tyrosine kinases and protein tyrosine phosphatases (PTPs). One subfamily of cytoplasmic PTPs, referred to as SHP (1), contains SH2, src homology 2, and Drosophila homology 2 (SH2, src homology 2; SHP, SH2 domain-containing PTP; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; EGF, epidermal growth factor; EpoR, erythropoietin receptor; GST, glutathione S-transferase; PI, phosphatidylinositol; Tyr(P), phosphotyrosine; SRE, serum-responsive element; PAGE, polyacrylamide gel electrophoresis; Luc, luciferase; PCR, polymerase chain reaction.

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** The abbreviations used are: PTP, protein tyrosine phosphatase; SH2, src homology 2; SHP, SH2 domain-containing PTP; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; EGF, epidermal growth factor; EpoR, erythropoietin receptor; GST, glutathione S-transferase; PI, phosphatidylinositol; Tyr(P), phosphotyrosine; SRE, serum-responsive element; PAGE, polyacrylamide gel electrophoresis; Luc, luciferase; PCR, polymerase chain reaction.

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Experimental Procedures

Materials and Cell Culture—MCF-7 (human breast carcinoma) and TRMP (canine kidney epithelial) cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Antibodies used were rabbit anti-SHP-1 polyclonal antibody generated as described previously (27), mouse anti-SHP-1 monoclonal antibody (anti-PTP 1C) (Transduction Laboratories), rabbit anti-human PDGF type β receptor polyclonal antibody (Upstate Biotechnology), mouse anti-PDGF type β receptor monoclonal antibody (Genzyme), rabbit anti-p85α polyclonal antibody (Santa Cruz Biotechnology),...
mouse anti-p85α monoclonal antibody (Transduction Laboratories), goat anti-GST polyclonal antibody (Pharmacia Biotech Inc.), and mouse anti-phosphotyrosine monoclonal antibody 4G10 (Upstate Biotechnology). Protein A-Sepharose CL-4B was obtained from Pharmacia Biotech Inc. Nicot cellulose membrane Hybond-C, anti-mouse IgG, horseradish peroxidase, and the nitrocellulose kit were purchased from Amersham Corp. Human recombinant platelet-derived growth factor BB (PDGF) was purchased from Upstate Biotechnology.

The peptides were synthesized and purified at the Biotechnology Research Institute (Quebec, Canada). Mass spectral analysis gave the expected molecular mass. The peptide sequences are shown in Table I, and the phosphoseryl sites are indicated.

**Construction and Expression of the PDGF Receptor and SHP-1 in MCF-7 Cells**—Human PDGF receptor β, wild type SHP-1, and catalytically inactive SHP-1 with a Cys65 to Ser mutation (SHP-1 C455S) were constructed into pRcCytomegalovirus vector as described previously (28). The other two catalytically inactive mutants, SHP-1 D421A (Asp421 to Ala) and SHP-1 C455A (Cys455 to Ala) (deletion of Cys65), were amplified by PCR and used as new templates for the secondary PCR. The primers used were GGAATTCATGCTGTCCCGTGGGTGG (primer 1, forward) with GGAGCCAATGGGCGCACCTCAGCTG (reverse), and GAGCTGGCCGCCTCATGGGTGCCAGTCG (forward) with GGGTGGACGCTTGGAGGACTGCGGT (primer 2, reverse). For construction of SHP-1 C455Ser (Ser455 deletion), primer 1 was forward with TGGGTGCGCTTGGCGAGCGATGTTGGCCTGC (reverse) and CATCGTGAAGAGGCGACAGACCTGTGCATC (forward) with primer 2 (reverse). The two PCR fragments encompassing the mutation were annealed with each other and extended, and the extended DNA fragments were used as new templates for the secondary PCR using primer 1 and primer 2 (in the first PCR) as primers. The secondary PCR product was digested with EcoRI and SalI, and the digested fragments were inserted into the mammalian expression vector pcDNA3 at the HindIII site by blunt-end ligation. The sequence and direction were confirmed by DNA sequencing.

The constructs mentioned above were transfected into MCF-7 cells by standard calcium phosphate co-precipitation technique, and clone cell lines overexpressing the PDGF receptor, SHP-1 or mutant SHP-1 C455S were isolated by selection in 400 μg/ml G418 and 418 and throughout the study.

TRPC cells that express stably transfected wild type PDGF receptor or its mutants PDGFR Y740F, Y751F, Y771F, Y1009F, and Y1021F were kindly provided by Dr. J. A. Cooper, Fred Hutchinson Cancer Research Center (29).

**Immunoprecipitation and Immunoblotting**—100–80% confluent cells growing in a 100-mm tissue culture dish were lysed in 1 ml of buffer A (50 mM β-glycerophosphate, pH 7.3, 2 mM EDTA, 1 mM EGTA, 5 mM β-mercaptoethanol, 100 mM NaCl, 1% Triton X-100, 0.2 mM Na3VO4, 0.1 μM microcin, 1.0 mM benzamide, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 1 μM pepstatin A, and 1 μM aprotinin). Cell lysates were clarified by centrifuging at 14,000 rpm for 10 min at 4 °C. One-fifth of a 50% slurry of protein A-Sepharose CL-4B was added to 1 ml of cell lysates and incubated for 30 min with gentle shaking. The precleared lysates were subjected to immunoprecipitation and immunoblotting as described previously (15). For Western blot analyses, one-fifth of the precipitates were subjected to SDS-PAGE gels and electrotransferred to nitrocellulose membranes. The membranes were probed with specific antibodies using the concentrations and conditions recommended by the manufacturers. Immune reactive protein bands were revealed by chemiluminescence with ECL detection according to the manufacturer’s instruction (Amersham Corp.).

In Vivo Peptide Competition—MCF-7 cells were permeabilized in 40 mM Hepes buffer, pH 7.4, with 10 mM MnCl2, 2 mM EGTA, 300 μM CaCl2, 1 mM 2-mercaptoethanol, and 285 μg/ml α-lyso phosphatidylcholine, palmitoyl (Mire’s buffer), either alone or containing 1.4 mM peptides, on ice for 1 min. Cells were warmed for 2 min at 37 °C before stimulation with 50 ng/ml PDGF. Cells were then lysed with buffer A as described above.

**GST Fusion Proteins and in Vitro Binding Assays**—The DNA fragment encoding amino acids 1–213 of SHP-1 was amplified by PCR and inserted into pGEX-2T (Pharmacia). The fusion protein and GST alone were expressed in Escherichia coli strain DH5α and freshly prepared for the in vitro binding experiments. For binding assays, glutathione-Sepharose beads with approximately 1 μg of bound GST or GST fusion protein were incubated at 4 °C for 2 h with 1 ml of cell lysates that were prepared from the cells grown in a 100-mm tissue culture dish to 70–80% confluence as described above. The beads were washed four times with ice-cold lysis buffer (buffer A), and one-fifth of the bound proteins were analyzed by SDS-PAGE and Western blotting.

**Far Western Blotting**—p85 was immunoprecipitated from PDGF-stimulated or unstimulated MCF-7 cells under the conditions as described above (see also Ref. 15). The complexes were dissociated by boiling the immunoprecipitates in SDS sample buffer, and the eluted proteins were separated by SDS-PAGE gel and transferred to nitrocellulose membrane. After blocking with 5% milk overnight at 4 °C, the membrane was incubated with 1 μg/ml GST fusion protein containing the SH2 domains of SHP-1 for 2 h and then subjected to Western blotting using the polyclonal anti-GST antibody and appropriate secondary antibody (15).

**Luciferase Activity Assay**—Transfection of the luciferase reporter gene (SRE-Luc) and assay of luciferase activity were described previously (15).

**RESULTS**

**SHP-1 Associates with p85 and PDGF Receptor**—As MCF-7 cells, a breast carcinoma cell line, express both SHP-1 and the PDGF receptor, we initially employed this cell line for experiments. To address the potential role of SHP-1 in PDGF receptor-mediated signaling, we first performed immunoprecipitation with anti-SHP-1 antibody in cells stimulated with PDGF. Proteins that were co-precipitated with SHP-1 were examined by Western blot analysis with anti-phosphotyrosine antibody 4G10. As shown in Fig. 1A (lane 2), one intensified band and two faint bands with molecular masses of approximately 160–180, 80–90, and 65 kDa were detected. To clarify the identity of the associated proteins, we overexpressed the wild type and the catalytically inactive mutant SHP-1 C455S (28) in these cells. From various stable cell clones, two wild type SHP-1 and two mutants (SHP-1 C455S) were chosen from independent transfections for further characterization. These clones typically expressed a high and comparable level of SHP-1 or SHP-1 C455S (Fig. 1B). Interestingly, in the SHP-1-overexpressed cells (lane 4 in Fig. 1A), the intensity of the 80–90- and 65-kDa bands increased significantly, whereas the tyrosine-phosphorylated signal of 160–180-kDa band was substantially reduced in comparison with the parental cells (lane 2 in Fig. 1A). In cells transfected with the mutant SHP-1 C455S, all three bands became much stronger than those observed in the SHP-1-overexpressed cells, although the expression level of SHP-1 and mutant SHP-1 C455S in the cells were comparable (Fig. 1B).

The 65- and 160–180-kDa proteins were easily proved to be SHP-1 and the PDGF receptor, respectively, by immunoblotting the same samples with anti-SHP-1 and anti-PDGF receptor antibodies (Fig. 1C). To identify the nature of the 80–90-kDa protein, we probed the precipitates with antibodies to various candidate proteins. Eventually, it was revealed that the 80–90-kDa protein was the α type 85-kDa subunit (p85) of phosphatidylinositol 3-kinase (PI 3-kinase) (Fig. 1C). With previous results (28), the overexpressed SHP-1, when tyrosine-phosphorylated in response to PDGF stimulation (lane 4 in Fig. 1A), was rapidly dephosphorylated by its own activity. Retention of the high level of tyrosine phosphorylation on p85 and the PDGF receptor in the SHP-1 C455S transfected cells (lane 6 in Fig. 1A) implies that these two proteins, particularly the PDGF receptor, might be substrates of SHP-1 in PDGF-activated signaling pathway.

To demonstrate further the interaction of these proteins, reciprocal experiments were performed, i.e. the cell lysates were subjected to immunoprecipitation with anti-PDGF receptor and anti-p85 antibodies, respectively, and the immunoprecipitates were immunoblotted with anti-SHP-1 antibody. As expected, SHP-1 was detected in both the PDGF receptor and p85 immunoprecipitates produced from PDGF-stimulated cells (Fig. 1, D and E).

**SHP-1 Independently Associates with Both the PDGF Receptor and p85**—We have shown that SHP-1 was co-immunoprecipitated with both the tyrosine-phosphorylated PDGF receptor
and p85. However, it is not clear whether SHP-1 binds independently to the PDGF receptor and p85 or the three proteins associate together through a high order complex. This is possibly due to the presence of the SH2 domains in both SHP-1 and p85, and the induction of tyrosine-phosphorylation following PDGF stimulation on these proteins, resulting in their utilization as adaptors to interact with each other. To investigate the nature of the association between SHP-1, the PDGF receptor, and p85, we used TRMP cells that stably express transfected wild type or mutant PDGF receptors lacking various tyrosine phosphorylation sites. As shown in Fig. 2A, p85 could not be co-immunoprecipitated with the receptor mutants utilized, either PDGFR-Y740F or PDGFR-Y751F. This confirms the finding that the association of p85 with the PDGF receptor is through both the phosphorylated Tyr740 and Tyr751 residues on the receptor (29, 30). However, mutation of these two binding sites on the receptor did not abolish the co-precipitation of SHP-1 with the mutant PDGF receptor (Y740F) (Fig. 2B).

These results indicate that SHP-1 can directly associate with the PDGF receptor without the mediation of p85. The interaction of SHP-1 with p85 was also examined in the TRMP cells. Although p85 was not co-precipitated with the mutant PDGF receptors as shown in Fig. 2A, this protein could be co-precipitated with SHP-1 in TRMP cells expressing the mutant PDGF receptor (Fig. 2C). The amount of SHP-1 precipitated by p85 in cells expressing the mutant receptor was as high as that detected in cells expressing the wild type receptor (Fig. 2C). This result, taken together with those in Fig. 2, A and B, indicates that SHP-1 can associate with p85 without the binding of p85 to the PDGF receptor. Thus, SHP-1 likely associates directly with the PDGF receptor and p85, respectively. Further evidence for the direct association of SHP-1 with the PDGF receptor and p85 will be presented below.

Whether p85 can be tyrosine-phosphorylated as a result of activation of the PDGF receptor has not been clearly established. To demonstrate directly tyrosine phosphorylation of p85 in response to PDGF stimulation, p85 was immunoprecipitated with anti-p85 antibody. The immunoprecipitates were probed with anti-phosphotyrosine antibody. Fig. 2D shows that p85, as with the co-precipitated PDGF receptor, was tyrosine-phosphorylated in response to PDGF stimulation. A reciprocal experiment was also performed in which cell lysates were immunoprecipitated by anti-phosphotyrosine antibody and then examined with an anti-p85 antibody. Again, p85 was proved to be precipitated by anti-phosphotyrosine antibody (data not shown). Interestingly, in response to PDGF stimulation, p85 even can be tyrosine-phosphorylated in TRMP cells expressing only the mutant PDGFR-Y740F (Fig. 2D), suggesting that tyrosine phosphorylation of p85 does not require its binding to the receptor, although its association with the PDGF receptor increased the intensity of its tyrosine phosphorylation (Fig. 2D).
and p85, between SHP-1 and the PDGF receptor and between SHP-1 and the PDGF receptor—
To investigate the mechanism of interaction between either unstimulated or PDGF-stimulated cells (Fig. 3B), proteins were precipitated by the GST protein alone or immobilized on glutathione-Sepharose beads, respectively. The beads were incubated with the lysates of unstimulated or PDGF-stimulated MCF-7 cells. The proteins precipitated by the immobilized proteins were analyzed by immunoblotting with anti-phosphotyrosine, anti-PDGF receptor, or anti-p85 antibodies, respectively. As shown in Fig. 3A, only two major bands were detected in the anti-phosphotyrosine immunoblots of the GST-SHP-1 SH2 precipitates from PDGF-stimulated cells but not from unstimulated cells. The much lower intensity band with a molecular mass of approximately 180 kDa directly interacts with the SH2 domain of p85 and with the PDGF receptor. This protein is likely the PDGF receptor, which has been shown previously to bind to the N-terminal SH2 domain of SHP-1 as described above, we used far Western blot analysis. p85 immunoprecipitates were transferred to nitrocellulose and the membranes were incubated with a fusion protein containing the SH2 domains of SHP-1 (GST-SHP-1 SH2) and then probed with anti-GST antibody. As shown in Fig. 3B, GST-SHP-1 SH2 directly bound to the p85 band immunoprecipitated from the PDGF-stimulated MCF-7 cells but not to that from the unstimulated cells. This experiment also revealed that a protein in the p85 immunoprecipitates with a molecular mass of approximately 180 kDa directly interacts with the SH2 domain of p85. In addition, we found that p85 was still co-precipitated with the mutant SHP-1 Y538F and Y543F (data not shown). Since Tyr538 and Tyr 543 are the major tyrosine phosphorylation sites in SHP-1 (28, 35, 36), mutation of these sites should disturb potential binding of the SH2 domain of p85 to SHP-1.

Taken together, these results strongly suggest that p85 directly associates with SHP-1 through the binding of the p85 subunit to the SH2 domain of SHP-1, and not conversely through the SH2 domain of p85 with tyrine-phosphorylated SHP-1. Since SHP-1 was detected independently to be associated solely with the PDGF receptor and p85, i.e. no other apparent tyrosine-phosphorylated proteins were detected in the in vitro binding experiment using GST fusion protein of the SH2 domain of SHP-1 (Fig. 3A), it is likely that SHP-1 also directly interacts with the PDGF receptor.

SHP-1 Associates with the PDGF Receptor and p85 via Its N-terminal SH2 Domain—Since SHP-1 contains two SH2 domains and each SH2 domain can bind specific tyrosine-phosphorylated proteins (17, 31), we were interested in assessing which SH2 domain of the enzyme is involved in the interaction with these two proteins. We therefore performed in vitro peptide competition experiments based on two tyrosine-phosphorylated peptides specifically binding to the individual SH2 domains of SHP-1. One phosphopeptide, EpoR Tyr(P)429, contains the sequence surrounding Tyr429 in the erythropoietin receptor, which has been shown previously to bind to the N-terminal...
SH2 domain of SHP-1 (17). The other peptide, FcYRIIB1 Tyr(P)309, contains the sequence surrounding Tyr309 in FcYRIIB1 ITIM motif. This tyrosine-phosphorylated peptide specifically binds to the C-terminal SH2 domain of SHP-1 (31). The other tyrosine-phosphorylated peptides used are listed in Table I, including PDGFR Tyr(P)1009 surrounding the SH2-binding site (32, 33) and PDGFR Tyr(P)771 surrounding the GTPase activating protein-binding site (29, 30). Three other peptides, Tyr(P)368, Tyr(P)580, and Tyr(P)607 designed from the three reported tyrosine-phosphorylated sites on p85 (34), were also included. MCF-7 cells stably overexpressing SHP-1 were permeabilized to allow entrance of phosphopeptides prior to stimulation with PDGF. As shown in Fig. 4A, the tyrosine-phosphorylated peptide EpoR Tyr(P)429 completely blocked the co-precipitation of SHP1 with the PDGFR receptor. The other three tyrosine-phosphorylated peptides, FcYRIIB1 Tyr(P)309, PDGFR Tyr(P)1009, and PDGFR Tyr(P)771, did not significantly affect the co-precipitation. Similarly, the tyrosine-phosphorylated peptide EpoR Tyr(P)429 also effectively abolished the co-precipitation of SHP1 with tyrosine-phosphorylated p85 (Fig. 4B), whereas the other four phosphorylated peptides, FcYRIIB1 Tyr(P)309, Tyr(P)368, Tyr(P)580, and Tyr(P)607, did not display any detectable effect on the co-immunoprecipitation. This experiment also shows that the association of SHP-1 with p85 is not very likely to occur through the binding of the SH2 domain of SHP-1 to any of the three known tyrosine phosphorylation sites previously identified on p85 following insulin stimulation (34). The effect of peptide EpoR Tyr(P)429 on blocking the co-precipitation of SHP1 with both the PDGFR receptor and p85 is phosphorylation-dependent. The non-tyrosine-phosphorylated EpoR Tyr(P)429 did not display its competition in the co-immunoprecipitation. These results clearly demonstrate that SHP-1 associates with both the PDGFR receptor and p85 through its N-terminal SH2 domain.

Effect of Overexpressed SHP-1 on the PDGF-stimulated c-fos Promoter Activation—To investigate the role of SHP-1 in PDGF-activated signaling, we examined the effect of wild type and catalytically inactive mutants of SHP-1 on PDGF-induced activation of the c-fos promoter. The c-fos promoter contains a well characterized serum-responsive element (SRE) whose activity can be stimulated upon mitogenic activation of appropriate receptors. The plasmid construct containing the c-fos promoter-driven luciferase gene (SRE-Luc) was co-transfected with SHP-1 or its mutants, SHP-1 C455S, SHP-1 D421A, and SHP-1Δ (455–461), into TRMP cells. It has been shown that all three mutants (SHP-1Δ C455S, SHP-1 Δ D421A, and SHP-1Δ (455–461)) have very little or no catalytic activity (37). The mutations in these phosphatases, however, do not affect the affinity of the SH2 domain binding to tyrosine-phosphorylated proteins, and additionally, mutant SHP-1 C455S and SHP-1 D421A retain their ability to interact with substrates (37). Accordingly, these mutants would be predicted to act as biochemically dominant negative modulators of SHP-1 in vivo. As shown in Fig. 5, the luciferase activity driven by SRE promoter was induced 2.9-fold following PDGF stimulation in the control TRMP cells (which were transfected by SRE-Luc and vector alone), whereas overexpression of wild type SHP-1 caused an induction of only 2.1-fold, resulting in a 30% reduction in the PDGF-stimulated luciferase activity. In contrast, expression of all three catalytically inactive mutants enhanced PDGF induction of the c-fos promoter-driven luciferase expression by approximately 20–45%. Moreover, the inhibitory effect of the overexpressed wild type SHP-1 on the PDGF-stimulated c-fos promoter-driven luciferase activity could be blocked by overexpressing the catalytically inactive SHP-1 (SHP-1 C455S) (data not shown). Taken together, these results suggest that SHP-1 negatively regulates PDGF receptor-mediated signaling in the SRE-controlled transcription.

**DISCUSSION**

As is the case with other receptor protein tyrosine kinases, upon binding of its ligand, the PDGF receptor is activated via dimerization, resulting in autophosphorylation of multiple tyrosine residues within its intracellular domain. The autophosphorylated PDGF receptor provides docking sites to recruit
specific cellular SH2 domain-containing proteins, many of which have been identified, including the SH2 domain-containing PTP, SHP-2 (32, 33). In our immunoprecipitation experiments, we found that SHP-1, structurally related to SHP-2, also associated with the PDGF receptor in response to ligand stimulation in the non-hematopoietic MCF-7 and TRMP cell lines. Notably, the p85 subunit of PI 3-kinase was also co-precipitated with SHP-1. To address the nature of these interactions, we took the advantage of available TRMP cell lines transfected with mutant PDGFR Y740F and PDGFR Y751F which are unable to bind to p85. We demonstrated that although no p85 was associated with the mutant PDGF receptor, SHP-1 was still co-precipitated with the mutant PDGF receptor, suggesting that SHP-1 associates with the PDGF receptor without p85 mediation. Additionally, in the cells expressing only the mutant PDGF receptor, SHP-1 could associate with p85 without the binding of p85 to the PDGF receptor. These results suggest that SHP-1 independently associates with both the PDGF receptor and p85. The in vitro binding experiments further showed that SHP-1 associated with the PDGF receptor and p85 through its SH2 domains, whereas peptide competition experiments subsequently specified that the N-terminal domain of SHP-1 was responsible for both of these interactions. The binding sites for SHP-1 on both the PDGF receptor and p85, however, are presently unknown. We attempted to identify the binding sites by two approaches, phosphopeptide competition and using PDGF receptor mutants for co-immunoprecipitation. Experiments with phosphopeptide competition in co-precipitation (Fig. 4A) likely excluded the possibility that SHP-1 bound to Tyr<sup>1009</sup>, a binding site for the structurally
related SHP-2 (32, 33). The experiments also excluded the binding of SHP-1 to Tyr771, a GTPase activating protein-binding site (29, 30). We have used PDGF receptor mutants PDGFR Y740F, Y751F, Y771F, Y1009F, and Y1021F in co-immunoprecipitation with SHP-1. All these mutations were unable to interfere with the co-precipitation of the receptors with SHP-1 (Fig. 2B), suggesting that the SH2 domain of SHP-1 does not bind to these phosphorylation sites. Similarly, the SH2 domain of SHP-1 does not appear to bind to the reported phosphorylation sites of Tyr368, Tyr580, and Tyr607 in p85 (34) as the association of SHP-1 with p85 in co-immunoprecipitation (Fig. 4B).

The function of SHP-1 in PDGF receptor signaling was studied by assessing the effect of wild-type SHP-1 and its dominant negative mutants on PDGF-induced c-fos promoter activation. In TRMP cells, the overexpression of wild-type SHP-1 markedly inhibited the response of the c-fos promoter to PDGF stimulation, whereas the expression of biochemically dominant negative mutants increased the response. These results suggest that SHP-1 can negatively regulate PDGFR-mediated signaling at least in SRE-regulated transcription in TRMP cells. However, in other cell lines, particularly CCL39 cell, it was reported that overexpression of SHP-1 did not affect PDGF-induced DNA synthesis (38). It appears that, depending on the cell type, its compartmentalization, and its targeting molecules, SHP-1 could play different roles in growth factor-activated pathways, such as a negative role observed here in the PDGF-induced c-fos promoter activation, a positive role reported in EGF-stimulated pathway (15), or no apparent effect on PDGF-stimulated DNA synthesis (38) and on EGF-stimulated mitogen-activated protein kinase activation and Elk-1 transcription (39).

At present, the mechanism(s) by which SHP-1 positively or negatively regulates various growth factor-activated pathways is largely unknown. In our co-immunoprecipitation experiments, we observed that overexpression of wild-type SHP-1 substantially reduced the intensity of tyrosine phosphorylation on the PDGF receptor, whereas expression of the catalytically inactive mutant SHP-1 C455S dramatically increased the tyrosine phosphorylation of the receptor and p85. This result may suggest that SHP-1 targets the autophosphorylated PDGF receptor as its substrate and dephosphorylates the receptor on certain tyrosine residues, thus inhibiting the ligand-stimulated signaling, a mechanism similar to that found in cytokine receptor-mediated signalings where SHP1 negatively regulates these signal transduction pathways (16, 17). The intensity of tyrosine-phosphorylated p85 was also greatly increased in cells expressing catalytically inactive mutant SHP1 C455S. It is likely that SHP1 also targets p85 as a substrate. It is unknown whether dephosphorylation of p85 contributes the negative effect of SHP1 on the PDGF receptor relaying signaling.

Interestingly, SHP-2 can also positively or negatively regulate mitogen-stimulated pathways. The positive function of SHP-2 was reported in EGF, insulin, and PDGF-activated signal transductions (38–47). Likewise, it was also reported that SHP-2 had a negative role or was not required in PDGF receptor signaling (7, 39, 48–50). We also found that the dominant negative mutants of SHP-2 dramatically suppressed the EGF-stimulated signal pathway (44), but it had no effect on the PDGF-activated mitogenesis in TRMP cells. The seemingly conflicting reports on the function of SHP-1 and SHP-2 in growth factor receptor protein tyrosine kinase signaling are probably due to redundancy and convergence of multiple signals from the growth factor receptors and the existence of multiple substrates that may be differentially expressed and/or differentially regulated by these PTPs. Thus both SHP-1 and SHP-2 may have diversified functions in complicated and multiple signaling pathways.

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