The Novel Role of the C-terminal Region of SHP-2

INVIOLEMENT OF Gab1 AND SHP-2 PHOSPHATASE ACTIVITY IN Elk-1 ACTIVATION∗

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SHP-2, a nontransmembrane-type protein-tyrosine phosphatase that contains two Src homology 2 (SH2) domains, is thought to participate in growth factor signal transduction pathways via SH2 domain interactions. To determine the role of each region of SHP-2 in platelet-derived growth factor signaling assayed by Elk-1 activation, we generated six deletion mutants of SHP-2. The large SH2 domain deletion SHP-2 mutant composed of amino acids 198–593 (SHP-2-(198–593)), but not the smaller SHP-2-(399–593), showed significantly higher SHP-2 phosphatase activity in vitro. In contrast, SHP-2-(198–593) mutant inhibited wild type SHP-2 phosphatase activity, whereas SHP-2-(399–593) mutant increased activity. To understand these functional changes, we focused on the docking protein Gab1 that assembles signaling complexes. Pull-down experiments with Gab1 suggested that the C-terminal region of SHP-2 as well as the SH2 domains (N-terminal region) associated with Gab1, but the SHP-2-(198–593) mutant did not associate with Gab1. SHP-2-(1–202) or SHP-2-(198–593) inhibited platelet-derived growth factor-induced Elk-1 activation, but SHP-2-(399–593) increased Elk-1 activation. Co-expression of SHP-2-(1–202) with SHP-2-(399–593) inhibited SHP-2-(399–593)/Gab1 interaction, and the SHP-2-(399–593) mutant induced SHP-2 phosphatase and Elk-1 activation, supporting the autoinhibitory effect of SH2 domains on the C-terminal region of SHP-2. These data suggest that both SHP-2/Gab1 interaction in the C-terminal region of SHP-2 and increased SHP-2 phosphatase activity are important for Elk-1 activation. Furthermore, we identified a novel sequence for SHP-2/Gab1 interaction in the C-terminal region of SHP-2.

SHP-2 is a widely expressed protein-tyrosine phosphatase that has two tandem SH2 domain repeats at its N terminus (1). SHP-2 binds directly to growth factor receptors, including the PDGF and EGF receptors, in response to receptor stimulation with the corresponding ligand and undergoes tyrosine phosphorylation (2, 3). Accumulating evidence implicates SHP-2 as a positive regulator of ERK activity downstream from receptor-tyrosine kinases (4). Fibroblasts derived from SHP-2 exon 3−/− mice exhibit decreased ERK activity in response to EGF and PDGF. Following growth factor stimulation, SHP-2 is recruited through its SH2 domain directly to the EGF or PDGF receptor (5, 6). SHP-2 function is regulated by both intramolecular associations and binding of regulatory proteins (Fig. 1A). Schematically, SHP-2 consists of a phosphatase domain in the C terminus (PTP2 in Fig. 1A) and two tandem SH2 domains in the N terminus. It has been proposed that the SH2 domains maintain SHP-2 in an inactive conformation under basal conditions. Specifically, previous investigators found that deletion of the SH2 domains of SHP-2 protein enhanced phosphatase activity, and binding of tyrosine-phosphorylated peptides to the SH2 domains (presumably removing their inhibitory interaction with PTP2) increased SHP-2 catalytic activity (7, 8). The SHP-2 crystal structure provides additional support for this model (9), since it predicts that the N-terminal SH2 domain binds the phosphatase domain and directly blocks its active site.

In addition to SH2 domains, it is possible that the C-terminal portion of SHP-2 associates with other proteins via putative phosphotyrosine sites that may interact with SH2 domain (1). Interestingly, SHP-2 is activated by phospholipids that may be mediated by lipid interaction with the phosphatase domain, because a deletion mutant of SH2 domains still can respond to phospholipid stimulation (7). Recently, Xu et al. reported that SHP-2 co-localized with stress fibers at low cell densities and directly associates with F-actin through the phosphatase domain (10).

Gab1 is a docking protein that couples the EGF, PDGF, and Met receptor with multiple signaling proteins, including phosphatidylinositol 3-kinase, phospholipase Cγ, the adapter protein Crk, and SHP-2 (11–15). Especially, the association of Gab1 (Y637) with SHP-2 is critical for sustained ERK1/2 activation (16), suggesting that Gab1 and SHP-2 are important partners in cytoplasmic signaling. However, it remains unknown how SHP-2 phosphatase activity and SHP-2-Gab1 complexes mediate downstream events of growth factor receptors such as ERK1/2 and Elk-1 activation.

Based on previous data, there are three possible models by which SHP-2 may regulate ERK1/2 or Elk-1 activity. 1) Both SHP-2-Gab1 complex formation and SHP-2 phosphatase activity are critical for ERK1/2 and Elk-1 activation. 2) The N-terminal SH2 domains of SHP-2 associate with Gab1, which increases SHP-2 phosphatase activity and activates ERK1/2 and Elk-1 activity. 3) Association of Gab1 with SHP-2 is the only mechanism necessary to regulate ERK1/2 and Elk-1 ac-
Gab1 Associates with C-terminal Domain of SHP-2

ERK1/2 activity may be
tutively (17), a possible mechanism for SHP-2C/S inhibition of
tive (4, 7, 8), the actual involvement
of SHP-2 phosphate activity is not clear. Model 3 is supported
shing events such as Elk-1 activation. Structure function
alysis indicates a novel role for the C-terminal region of
SHP-2 in regulating Elk-1 activation by both mediating inter-
action with Gab1 and regulating SHP-2 phosphate activity.

EXPERIMENTAL PROCEDURES

Materials—[^32P]ATP was purchased from Amersham Biosciences. Raytide and pp60^src tyrosine kinase were from Calbiochem. Anti-
hemagglutinin (HA), polyclonal SHP-2 antibody (C-18), and Bcr (break-
point cluster region protein) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-Gab1 antibodies and the anti-phosphotyrosine
antibody 4G10 were purchased from Upstate Biotechnology, Inc. (Lake
Placid, NY). Antibodies against HisG and Xpress tag were from Invitro-
gene. Antibodies that specifically recognize phospho-ERK1/2 were from
New England Biolabs (Beverly, MA). Normal mouse and rabbit IgG and
anti-FLAG M2 antibodies were obtained from Sigma.

Cell Culture—Chinese hamster ovary (CHO) cells that overexpress
PDGF-R receptor were maintained in F-12 medium (Cellgro) supple-
mented with 10% fetal calf serum as previously described (18).

Plasmids and Transfection—pcDNA3 HA-Gab1 is a kind gift from
Dr. Neck (11), and we subcloned it into pcDNA3.1/His vector (Invitro-
gen) to add Xpress and His tag. pJ3-hSHP-2 WT (WT) and C459S
(SHP-2/C/S)), which abolishes SHP-2 phosphate activity, are kind
gifts from Dr. Saxton (19) and were also subcloned into pcDNA3.1/His
vector. For transient expression experiments, CHO cells were trans-
fected with Lipofectamine Plus (Invitrogen) as described previously
(20). pcDNA1.Bcr wild type was generated as described previously
(18). The N-terminal truncations of SHP-2 (SHP-2-(1–202) and SHP-2-
(1–411)) and the C-terminal truncations of SHP-2 (SHP-2-(198–593)
and SHP-2-(399–593)) were created by polymerase chain reaction from
SHP-2 wild type and subcloning into pcDNA3.1.hisC or pCMV-Tag2 vectors (Invitrogen). Phosphatase-inactive mutant forms of SHP-2-
(198–593) (SHP-2-(198–593)/S/S) and SHP-2-(399–593) (SHP-2-(399–
593)/C/S) were generated by polymerase chain reaction from SHP-2/C/S.
All plasmid constructs were verified by DNA sequencing.

Immunoprecipitation and Western Blot Analysis—After treatment
with reagents, the cells were washed with ice-cold PBS (−), harvested
in 0.5 ml of lysis buffer (50 mM sodium pyrophosphate, 50 mM NaF, 50
mm NaCl, 5 mM EDTA, 5 mM EGTA, 100 µM Na3VO4, 10 mM HEPES,
10 µg/ml leupeptin). After a flash-freezing on liquid nitrogen and thaw-
ing on ice, cells were scraped off the dish and centrifuged at 14,000 × g
(40 °C for 30 min), and protein concentration was determined using the
Bradford protein assay (Bio-Rad). For immunoprecipitation, cell lysates
were incubated with anti-HA, SHP-2, Bcr, Gab1, or Xpress antibody for
16 h at 40 °C as indicated and then incubated with 20 µl of protein A-
or protein G-Sepharose CL-4B (Amersham Biosciences) for 1 h on a
roller system at 40 °C. The beads were washed three times with 500 µl

Fig. 1. Schematic diagram of SHP-2 (A) and mutants (B) constructed by subcloning polymerase chain reaction products into mammalian expression vectors pcDNA3.1 or pCMV/Tag2 and named as indicated.
Effects of a catalytic inactive form of SHP-2 (SHP-2C/S) on PDGF-mediated ERK2 activation and on tyrosine phosphorylation and association with Gab1. 

A. PDGF β-receptor-overexpressing Chinese hamster ovary cells (CHO-PDGFR) were co-transfected with pcDNA3.1 vector alone, Xpress-SHP-2C/S, and HA-ERK2 and 24 h later washed with media and maintained in serum-free medium for 24 h. Cells were then treated with PDGF-BB (10 ng/ml) for the indicated times, and then cells were harvested in lysis buffer and immunoprecipitated with anti-HA antibody. The activated form of ERK2 was detected by Western blot analysis with phosphospecific ERK1/2 antibody for detecting ERK2 activity (A), No difference in the amount of immunoprecipitated ERK2 was found by Western blot analysis with anti-ERK2 (A, bottom). B, densitometric analysis of ERK2 activation. Results were normalized by arbitrarily setting the densitometry of control cells (time 0) to 1.0 (shown is the mean ± S.D.; n = 3; *, p < 0.05; **, p < 0.01). C, PDGF-induced SHP-2 phosphatase activity. CHO-PDGFR cells were stimulated with 10 ng/ml PDGF-BB for the indicated time periods. Cell lysates were immunoprecipitated with SHP-2 antibody, and the immunoprecipitates were then subjected to in vitro immune complex assay as described under “Experimental Procedures.” Data are the average of duplicate values and are representative of two independent experiments. D, CHO-PDGFR cells were co-transfected with plasmids for expression of HA-tagged Gab1 with the wild type SHP-2 or SHP-2C/S. Cells were growth-arrested for 16 h and stimulated with PDGF-BB (10 ng/ml) for the indicated times. HA-tagged Gab1 was immunoprecipitated, and immunoprecipitates were analyzed by immunoblotting (IB) with antibodies to SHP-2 (D, top), phosphotyrosine (4G10) (D, second from top), or HA (D, third from top). No difference in the amount of SHP-2 wild type or SHP-2 C/S expression was found by Western blot analysis with anti-SHP-2 (D, bottom). E, densitometric analysis of Gab1 association with SHP-2 wild type (open bar) or SHP-2C/S (solid bar). Results were normalized by arbitrarily setting the densitometry of the control SHP-2 wild type transfected cells (time 0) to 1.0. (shown is the mean ± S.D.; n = 3; **, p < 0.01).
A.

![Graph A](image)

B.

![Graph B](image)

C.

![Graph C](image)

FIG. 3. SHP-2-(399–593) mutant has no significant SHP-2 phosphatase activity but can increase SHP-2 wild type tyrosine phosphatase activity. A, CHO-PDGFR cells were transfected with vector alone, Xpress-tagged SHP-2-(1–593), SHP-2-(1–202), SHP-2-(1–411), SHP-2-(1–411), or SHP-2-(198–593). Each cell lysate was analyzed by immunoblotting (IB) with anti-Xpress antibody for detecting the expression of Xpress-tagged SHP-2 mutants. B, CHO-PDGFR cells were transfected with vector alone, SHP-2-(198–593), SHP-2-(198–593C/S), SHP-2-(399–593), or SHP-2-(399–593C/S) and 24 h later were washed with media and maintained in serum-free medium for 24 h. Cell lysates were immunoprecipitated with anti-Xpress antibody, and the immunoprecipitates were then subjected to in vitro immune complex phosphatase assay using 32P-labeled Tyr-Raytide as a substrate. The phosphatase activity was evaluated by the extent of 32P release from 32P-labeled Tyr-Raytide. Data are the average of duplicate values and are representative of three independent experiments. C, SHP-2-(399–593) and Gab1 increase SHP-2 wild type phosphatase activity, but SHP-2-(1–202), SHP-2-(1–411), SHP-2-(399–593C/S), SHP-2-(198–593), and SHP-2C/S inhibit PDGF-induced SHP-2 wild type phosphatase activity. CHO-PDGFR cells were co-transfected with vector alone, FLAG-SHP-2-(1–202), FLAG-SHP-2-(1–411), FLAG-SHP-2-(198–593), FLAG-SHP-2-(399–593), FLAG-SHP-2-(399–593C/S), nontagged (SHP-2C/S), nontagged (SHP-2C/S), or HA-Gab1 and Xpress-tagged SHP-2 WT and 24 h later washed with media and maintained in serum-free medium for 24 h. Cell lysates were immunoprecipitated with anti-Xpress antibody, and the immunoprecipitates were then subjected to in vitro immune complex phosphatase assay as described for B. Data are the average of triplicate values and are representative of three independent experiments (shown is the mean ± S.D.; **, p < 0.01).
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Figure 4. SHP-2 can form a dimer via the C-terminal region (aa 399–593), and Gab1 enhanced the C-terminal region (aa 399–593) dimer formation. A, the N-terminal region of SHP-2 (SHP-2(1–202) or SHP-2(1–411)) can associate with the C-terminal region of SHP-2 (SHP-2(399–593)). CHO-PDGFR cells were co-transfected with FLAG-tagged SHP-2(399–593) (lanes 1 and 2) or SHP-2(399–593C/S) (lanes 3 and 4), and Xpress-tagged SHP-2(1–202) (lanes 1 and 3) or Xpress-tagged SHP-2(1–411) (lanes 2 and 4). Cell lysates were immunoprecipitated with anti-Xpress. Immunoprecipitates were analyzed by immunoblotting with anti-FLAG antibody (top). No difference in the amount of FLAG-tagged SHP-2(399–593) expression was detected by Western blot analysis with anti-FLAG antibody (middle). Cell lysates were analyzed by immunoblotting with anti-Xpress antibody for detecting the expression of Xpress-tagged SHP-2(1–202) or SHP-2(1–411) (bottom). B, C-terminal region of SHP-2 (SHP-2(399–593)) can be dimerized. CHO-PDGFR cells were co-transfected with FLAG-tagged SHP-2(399–593) (lane 2) or SHP-2(399–593C/S) (lane 4) or vector (lanes 1 and 3), and Xpress tagged SHP-2(399–593) (lanes 1–4). Cell lysates were immunoprecipitated with anti-Xpress. Immunoprecipitates were analyzed by immunoblotting with anti-FLAG antibody (top). No difference in the amount of Xpress-tagged SHP-2(399–593) in immunoprecipitats was detected by Western blot analysis with anti-Xpress antibody (middle). C, Gab1 enhanced C-terminal region (aa 399–593) dimer formation. CHO-PDGFR cells were co-transfected with FLAG-tagged SHP-2(399–593) and Xpress-tagged SHP-2(399–593) with (lane 2) or without (lane 1) HA-Gab1. After 48 h of transfection, cell lysates were analyzed by immunoblotting with anti-Xpress antibody. Immunoprecipitates were analyzed by immunoblotting with anti-FLAG antibody for detecting the co-immunoprecipitation of FLAG-tagged SHP-2(399–593) (top). Cell lysates were analyzed by immunoblotting with anti-HA (second from the top), anti-FLAG (third from the top), or anti-Xpress (bottom) antibody for detecting the expression of HA-tagged Gab1, FLAG-tagged SHP-2(399–593), or Xpress-tagged SHP-2(399–593), respectively.

Catalytically inactive SHP-2 (SHP-2C/S) in PDGF-β-receptor-overexpressing CHO cells significantly inhibited PDGF-induced ERK2 activation.

It has been reported that after the binding of the SHP-2 SH2 domains to a tyrosine-phosphorylated target such as Gab1, SHP-2 adopts an "open" conformation associated with increased catalytic activity. Therefore, we also determined the time course of PDGF-induced SHP-2 tyrosine phosphatase activity (Fig. 2C). As shown in Fig. 2C, PDGF (20 ng/ml) increased SHP-2 tyrosine phosphatase activity ~2.5-fold, which reached a peak at 10 min and returned to the basal level within 30 min.

Catalytic Inactive Form of SHP-2 (SHP-2C/S) Constitutively Associates with Gab1—Since SHP-2 and Gab1 interaction is critical for ERK1/2 activation and SHP-2C/S inhibits EGF-induced ERK activation, we evaluated SHP-2C/S association with Gab1. As shown in Fig. 2, D and E, cells transfected with SHP-2C/S had elevated levels of Gab1 tyrosine phosphorylation and Gab1-SHP-2 complex in the serum-starved condition. After PDGF stimulation, there was a continuous rise in Gab1 tyrosine phosphorylation and continuous increase in the amount of SHP-2 associated with Gab1 as previously reported (Fig. 2, D and E). These data suggest that SHP-2C/S can trap Gab1 in a tyrosine-phosphorylated state. It has been reported that SHP-2-Gab1 complex formation is critical for ERK1/2 activation (16). In regard to the regulation of ERK1/2 activation by SHP-2-Gab1 complex formation, the finding that SHP-2C/S constitutively associates with Gab1 is incompatible with the inhibitory effect of SHP-2C/S on PDGF-induced ERK1/2 and Elk-1 activation. Therefore, we investigated further the role of Gab1/SHP-2 interaction and SHP-2 phosphatase activity in SHP-2 signaling.

Tyrosine Phosphatase Activity of SHP-2 Mutants and Effects on Wild Type SHP-2 Tyrosine Phosphatase Activity—To further characterize the role of SHP-2 tyrosine phosphatase activity, we constructed two SHP-2 C-terminal truncation mutants (SHP-2(1–202) and SHP-2(1–411)), two SHP-2 N-terminal deletion mutants (SHP-2(198–593) and (399–593)), and two catalytically inactive forms of SHP-2 (SHP-2(198–593C/S) and SHP-2(399–593C/S)) as indicated in Fig. 1B. To assay tyrosine phosphatase activity of SHP-2 mutants, we transfected PDGF β-receptor-overexpressing CHO cells with pcDNA3.1/His SHP-2(198–593), (399–593), (399–593C/S), (399–593C/S), and vector alone (Fig. 3A). SHP-2 proteins were immunoprecipitated with anti-Xpress antibody, and tyrosine phosphatase activity was measured by dephosphorylation of Raytide (21). As previously reported (7, 8), SHP-2(198–593), which is an N-terminal SH2 domain deletion mutant, possessed significantly higher SHP-2 phosphatase activity than wild type (Fig. 3B). However, SHP-2(399–593), which contains the C-terminal region of SHP-2, had no significant tyrosine phosphatase activity. In addition, a Cys to Ser point mutation at amino acid 459 (SHP-2(198–593C/S) and (399–593C/S)) resulted in no tyrosine phosphatase activity, as shown in Fig. 3B.

Because it has been reported that SHP-2 forms dimers in mouse fibroblasts (23), it is possible that one SHP-2 protein can regulate the SHP-2 phosphatase activity of its binding partner. Therefore, we determined whether SHP-2 mutants can regulate wild type SHP-2 tyrosine phosphatase activity. First, we co-transfected Xpress-tagged wild type SHP-2 with

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FIG. 5. Gab1 can associate with N-terminal (aa 1–202) and C-terminal (aa 399–593) regions of SHP-2. A, B (top), and C, CHO-PDGF cells were transfected with Xpress-tagged SHP-2(1–202) or SHP-2(399–593) (A), with Xpress-tagged SHP-2(1–411) or SHP-2(198–593) (B), or with FLAG-tagged SHP-2(399–593), or SHP-2(399–593C/S) (C) and 24 h later washed with media and maintained in serum-free medium for 24 h. Cell lysates were immunoprecipitated with anti-Gab1 antibody and assayed Xpress-tagged wild type SHP-2 tyrosine phosphatase activity with or without PDGF stimulation. As shown in Fig. 3C, lanes 1 and 2 in A–C) or rabbit IgG as a control (lanes 3 and 4 in A and B). Immunoprecipitates (IP) (lanes 1–4 in A and B and lanes 1 and 2 in C) and total cell lysates (TCL) (lanes 5 and 6 in A and B and lanes 3 and 4 in C) were analyzed by immunoblotting (IB) with anti-Xpress (A and B) or anti-FLAG (C) antibody. No difference in the amount of immunoprecipitated Gab1 was detected by Western blot analysis with anti-Gab1 antibody (A and B bottom).

FLAG-tagged SHP-2-(1–202), SHP-2-(1–411), SHP-2-(198–593), SHP-2-(399–593), SHP-2-(198–593C/S), SHP-2-(399–593C/S), or Gab1, immunoprecipitated with anti-Xpress antibody, and assayed Xpress-tagged wild type SHP-2 tyrosine phosphatase activity with or without PDGF stimulation. As shown in Fig. 3C, we found that SHP-2-(399–593) and Gab1 increased the basal level of SHP-2 tyrosine phosphatase activity (Fig. 3C, lanes 3, 6). Furthermore, SHP-2-(1–202), SHP-2-(1–411), and SHP-2-(399–593C/S) inhibited PDGF-induced SHP-2 tyrosine phosphatase activity similar to SHP-2-(202) (lanes 3, 6). Of note, a constitutively active form of SHP-2 (SHP-2(198–593)) significantly inhibited PDGF-induced SHP-2 tyrosine phosphatase activity (Fig. 3C, lane 4). No difference was observed in the expression level of wild type Xpress-tagged SHP-2 phosphatase activity with anti-Xpress antibody (data not shown). These data suggested that the N-terminal region of SHP-2 containing SH2 domains and mutants of Cys459 to Ser inhibited the phosphatase activity of the heterodimer wild type SHP-2.

SHP-2 Can Form a Dimer through the C-terminal Region of SHP-2—It has been reported that after the binding of the SH2 domains to tyrosine-phosphorylated targets such as Gab1, the SHP-2 adopts an “open” conformation and becomes catalytically active. We also found that the C-terminal regions of SHP-2-(399–593) and SHP-2-(399–593C/S) are able to associate with SH2 domains (SHP-2(1–202) and -(1–411)) (Fig. 4A).

Since SHP-2-(399–593) increased and SHP-2-(399–593C/S) decreased wild type SHP-2 tyrosine phosphatase activity, we evaluated whether SHP-2-(399–593) can associate with the C-terminal region of SHP-2 directly as a dimer. We co-transfected Xpress-tagged SHP-2-(399–593) (lanes 1–4) with FLAG-tagged SHP-2-(399–593) (lane 2) or FLAG-tagged SHP-2-(399–593C/S) (lane 4), immunoprecipitated with anti-Xpress antibody, and measured the association of SHP-2-(399–593) or -(399–593C/S) by anti-FLAG antibody. As shown in Fig. 4B, FLAG-tagged SHP-2-(399–593) or SHP-2-(399–593C/S) was co-immunoprecipitated with SHP-2-(399–593), suggesting a possible mechanism of SHP-2 dimerization through the C-terminal region of SHP-2.

Furthermore, we also evaluated the effect of Gab1 on this SHP-2 dimerization through the C-terminal region of SHP-2. We performed triple co-transfection experiments of Xpress-tagged SHP-2-(399–593) and FLAG-tagged SHP-2-(399–593C/S) and either vector (lane 1) or HA-Gab1 (lane 2). As shown in Fig. 4C, expression of Gab1 significantly increased the association of Xpress-tagged SHP-2-(399–593) with FLAG-tagged SHP-2-(399–593) (top panel). These data suggest that Gab1 enhances the ability of SHP-2-(399–593) to form dimers.

Gab1 Can Interact with the SH2 Domain and C-terminal Tyrosine Phosphatase Domain of SHP-2—Previous investigators have shown that Gab1 associates with SHP-2 via the N-terminal SH2 domain (23). We also found that SHP-2-(1–202) and -(1–411), which contain the N-terminal SH2 domain, were able to form a complex with Gab1 by coimmunoprecipitation study with anti-Gab1 antibody (Fig. 5, A and B). We determined that the deletion mutant SHP-2-(198–593), in which the N-terminal SH2 domain and a significant part of the C-terminal SH2 domain of SHP-2 is deleted, did not associate with Gab1 as previously reported (23). However, Gab1 was able to associate with the smaller construct SHP-2-(399–593), com-
posed of C-terminal SHP-2 (Fig. 5A). Interestingly, SHP-2-(399–593C/S) also can associate with Gab1, and no significant difference between the association of Gab1 with SHP-2-(399–593) and SHP-2-(399–593C/S) was observed (Fig. 5C). Equal amounts of Gab1 protein were present in Gab1 immunoprecipitates (data not shown). These results indicate that Gab1 is able to interact with the C-terminal region of SHP-2 as well as with SHP-2 SH2 domains and that the mutation of Cys459 to Ser did not affect Gab1 interaction with the C-terminal region of SHP-2.

Gab1 Can Interact with the C-terminal Region of the SHP-2 Tyrosine Phosphatase Domain—As shown in Fig. 5A, Gab1 was able to associate with SHP-2-(399–593) but not with SHP-2-(198–398). These data suggest that the domain of amino acids 198–398 of SHP-2 has an inhibitory effect on Gab1/SHP-2-(399–593) association. However, it is also possible that Gab1/SHP-2-(399–593) interaction is artificial, caused by the exposure of new surfaces by the deletion of aa 198–398. The usual way to determine the association of Gab1/SHP-2 is to mutate the binding site for Gab1 in SHP-2-(399–593). However, since the binding site of Gab1 is in the SHP-2 phosphatase domain, the mutation in SHP-2-(399–593) may change the SHP-2 phosphatase activity and also the protein structure of SHP-2. Therefore, to determine whether Gab1 is able to interact with C-terminal region of SHP-2-(399–593) in wild type SHP-2, we performed a competitive assay to evaluate Gab1 association with SHP-2-(399–593) as follows.

The competitive assay took advantage of our findings that Bcr could associate with SHP-2 at two different sites similar to Gab1. Specifically, Bcr binds to the SH2 domain in SHP-2-(1–202) and to the C-terminal region of SHP-2-(399–593). Second, overexpression of Bcr disrupted PDGF-induced Gab1/SHP-2 association (data not shown). In addition, as shown in Fig. 6A, the association of Gab1 with SHP-2C/S was disrupted by the expression of Bcr. These data suggested the presence of competitive binding of Gab1 and Bcr to SHP-2. Finally, to investigate the SHP-2 domain required for Gab1/SHP-2 disruption induced by Bcr, we performed triple co-transfection experiments of Xpress-tagged SHP-2-(1–202) or SHP-2-(399–593), HA-tagged Gab1, and either vector or Bcr. We found that Bcr disrupted the association between Gab1 and SHP-2-(399–593) but increased the interaction between Gab1 and SHP-2-(1–202) (Fig. 6B and C). These data suggested that the C-terminal region of SHP-2-(399–593) contains the domains required for Bcr to disrupt the SHP-2/Gab1 interaction. These data also suggested that Gab1 is able to associate with the C-terminal region of SHP-2-(399–593) even in full-length SHP-2, and this is not due to the artificial exposure of new surfaces by the deletion of aa 198–398.

The N-terminal Region of SHP-2 (aa 1–202 and 1–411) Inhibits but the C-terminal Region (aa 399–593) Stimulates Elk-1 Transcription Activity—To determine the individual roles of each SH2 domain and the C-terminal region of SHP-2 on downstream events in PDGF signaling, we investigated the effect on
FIG. 7. Effects of SHP-2 mutants on PDGF-induced Elk-1 activation. The PathDetect trans-reporting system was used for detecting Elk-1 activity in CHO-PDGFR cells. CHO-PDGFR cells were cotransfected with SHP-2WT (A), SHP-2C/S (B), SHP-2-(1–202) (C), SHP-2-(1–411) (D), SHP-2-(198–593) (E), SHP-2-(399–593) (F), SHP-2-(198–593C/S) (G), or SHP-2-(399–593C/S) (H) and reporter plasmid pFR-Luc along with Ga4 fusion expression vectors containing Elk-1 and pRL-CMV (Promega) as luciferase control reporter vector with or without PDGF-BB (20 ng/ml) stimulation. After stimulation with PDGF for 16 h, a luciferase assay was performed.
PDGF-induced Elk-1 transcription activity of SHP-2 wild type (SHP-2 WT), a catalytically inactive form of SHP-2 (SHP-2C/S), and SHP-2 deletion mutants. PDGF β-receptor-overexpressing CHO cells were co-transfected with SHP-2 wild type, SHP-2 mutants (SHP-2(1–202), SHP-2(1–411C/S), and SHP-2(399–593C/S)) and reporter plasmid pFR-Luc along with Gal4 fusion expression vectors containing Elk-1 and pRL-CMV (Promega) as luciferase control reporter vector. After transfection for 48 h, cells were applied to a luciferase assay as described under “Experimental Procedures.”

To study the role of phosphatase activity, we generated C459S mutations in SHP-2(198–593) (SHP-2(198–593C/S)) and SHP-2(399–593C/S) and determined the effect of these two mutants on PDGF-induced Elk-1 activation. As shown in Fig. 7G, SHP-2(198–593C/S) had no effect on PDGF-induced Elk-1 activation. In contrast to SHP-2(399–593), SHP-2(399–593C/S) did not increase Elk-1 activity but significantly inhibited PDGF-induced Elk-1 activation to a small extent (Fig. 7H). These data suggest a critical role of Cys459 in regulating PDGF-induced Elk-1 activation.

SHP-2(1–202) or SHP-2(1–411) Fragment Containing N-terminal SH2 Domain Inhibits SHP-2(399–593)-induced Elk-1 Activity—Since self-association of the N-terminal region of SHP-2 with the phosphatase domain inhibits tyrosine phosphatase activity, we determined whether SHP-2(1–202) or (1–411) inhibits the interaction between SHP-2(399–593) and Gab1.
Gab1 Associates with C-terminal Domain of SHP-2

and Gab1. As shown in Fig. 8A, FLAG-tagged SHP-2-(399–593) was co-immunoprecipitated with Gab1, and N-terminal SH2 domain SHP-2 fragments (SHP-2-(1–202) and -(1–411)) disrupted this Gab1 and SHP-2 phosphatase domain interaction.

Since we found that SHP-2-(1–202) and -(1–411) inhibited PDGF-induced Elk-1 activity (Fig. 7), we evaluated the effect of SHP-2-(1–202) or SHP-2-(1–411) on SHP-2-(399–593)-induced Elk-1 activation. Cotransfected SHP-2-(399–593) with SHP-2-(1–202) or SHP-2-(1–411) and determined Elk-1 transcriptional activity by reporter gene assay. SHP-2-(399–593) induced Elk-1 activity dose-dependently (open squares), whereas co-transfection of SHP-2-(1–202) or SHP-2-(1–411) significantly inhibited SHP-2-(399–593)-induced Elk-1 transcription activity (Fig. 8, B and C).

Furthermore, to determine whether Gab1 binding modulates SHP-2 phosphatase activity, we cotransfected the cells with Xpress-tagged SHP-2 wild type (Fig. 8D, lanes 1–6) and FLAG-tagged SHP-2-(399–593) (lanes 2–4) or FLAG-tagged SHP-2-(1–202) (lanes 3 and 5) or FLAG-tagged SHP-2-(1–411) (lanes 4 and 6). After 48 h of transfection, each cell lysate was immunoprecipitated with anti-Xpress antibody, and Xpress-tagged SHP-2 wild type phosphatase activity was determined as described under “Experimental Procedures.” As shown in Fig. 8D, we found that SHP-2-(399–593) increased Xpress-tagged SHP-2 wild type phosphatase activity (lane 2), as we have shown in Fig. 3C. However, co-transfection of SHP-2-(1–202) (lane 3) or SHP-2-(1–411) (lane 4) significantly decreased SHP-2-(399–593)-induced Xpress-tagged SHP-2 wild type phosphatase activity. An equal amount of Xpress-tagged SHP-2 wild type expression was found by Western blot analysis with anti-Xpress antibody (data not shown). These data suggested a possible functional role for SHP-2 SH2 domains (aa 1–202 or 1–411) in the inhibition of the C-terminal region of SHP-2 (SHP-2-(399–593))-induced SHP-2 phosphatase and Elk-1 activation.

**DISCUSSION**

The major finding of this study is that both Gab1 association with SHP-2-(399–593) and SHP-2 phosphatase activity are critical for the regulation of downstream events activated by growth factor receptors such as the PDGFB receptor (Fig. 9A). In addition, we found that the autoinhibition of SHP-2 phosphatase activity by SH2 domains also prevents association of Gab1 and SHP-2-(399–593) and subsequent PDGF- or SHP-2-(399–593)-induced SHP-2 phosphatase and Elk-1 activation.

We summarized our data in Table I, based on the characteristic features of Gab1 association and SHP-2 phosphatase activity. SHP-2-(399–593) can associate with Gab1 and increase wild type SHP-2 phosphatase activity, although SHP-2-(399–593) itself has no phosphatase activity. SHP-2C/S and SHP-2C/S can associate with Gab1 but cannot increase SHP-2 phosphatase activity and instead inhibit PDGF-induced wild type SHP-2 phosphatase activity. SHP-2-(198–593) (SH2 domain deletion mutant) shows high SHP-2 phosphatase activity but cannot associate with Gab1 and therefore cannot increase Elk-1 activation. Only SHP-2-(399–593) was able to increase Elk-1 activation (Fig. 7). The disruption of SHP-2-(399–593) and Gab1 interaction by co-expression of SHP-2-(1–202) or -(1–411) significantly inhibited SHP-2 phosphatase and Elk-1 activation induced by SHP-2-(399–593) and probably also by PDGF stimulation (Fig. 8). These data support an essential role for both Gab1 association with SHP-2-(399–593) and SHP-2 phosphatase activity in mediating Elk-1 activity (Table I).

Our data suggest that both Gab1-SHP-2 complex formation and SHP-2 tyrosine phosphatase activity are important for PDGF-induced Elk-1 activation. Specifically, 1) SHP-2-(399–593) mutant associated with Gab1 and increased wild type SHP-2 tyrosine phosphatase and Elk-1 activity; 2) SHP-2-(198–593) mutant had significantly high SHP-2 phosphatase activity but inhibited PDGF-induced Elk-1 activation; 3) SHP-2C/S and SHP-2-(399–593C/S) mutants associated with Gab1 but inhibited PDGF-induced SHP-2 phosphatase and Elk-1 activity; 4) the association of SHP-2-(399–593) with Gab1 was inhibited by co-transfection of SHP-2-(1–202) or SHP-2-(1–411), and SHP-2-(399–593)-mediated SHP-2 phosphatase and Elk-1 activation was inhibited by co-transfection of SHP-2-(1–202) and -(1–411). Our results are in agreement with a previous study in which mutation of the SHP-2 binding site of Gab1 inhibited growth factor-induced ERK1/2 activation (24).

Based on this study as well as previous work from other investigations (1, 16, 23, 24), we propose a scheme for PDGF-mediated SHP-2 activation leading to Elk-1 activation (Fig. 9). A novel aspect of this model is the significant role of C-terminal region of SHP-2-(399–593) to activate Elk-1. As shown in Fig. 9A, the N-terminal SH2 domain binds with the phosphatase domain and directly blocks its catalytic site (Fig. 9A, top). Interaction of N-terminal SH2 with tyrosine-phosphorylated ligand, such as Gab1 and PDGFR receptor, disrupts its phosphatase recognition surface and generates an open (“active”) state of SHP-2 tyrosine phosphatase activity (step 1) (9). In the current study, we found that the N-terminal domain of SHP-2 also inhibits Gab1 association with the C-terminal region of SHP-2 (Figs. 8 and 9A, middle, step 1). SHP-2 forms a dimer and thereby activates SHP-2 phosphatase activity and Gab1 association with the C-terminal region of SHP-2 (Fig. 9A, bottom, step 2). The combination of Gab1 association with the C-terminal region of SHP-2 and activating SHP-2 phosphatase activity are both critical for PDGF-induced Elk-1 activation (Fig. 9A). As shown in Fig. 9B, SHP-2C/S and SHP-2-(399–593C/S) can form a dimer (Fig. 4B) and associate with Gab1 (Fig. 2D). However, SHP-2C/S and SHP-2-(399–593C/S) inhibit PDGF-induced SHP-2 phosphatase activity (Fig. 3C) and Elk-1 activation (Fig. 7, B and H). In contrast, SHP-2-(198–593) (Fig. 9C) has SHP-2 phosphatase activity but is unable to interact with Gab1 (Fig. 5B) and inhibit PDGF-induced Elk-1 activation (Fig. 7E). Shi et al. (23) have reported that in fibroblasts, which express a deletion of 65 amino acids in the N-terminal SH2 SH2-246–110 domain EGF-induced Ras, Raf-1, and ERK activation were significantly decreased although such a deletion at the N-terminal produces a constitutively active form of SHP-2 (7, 8). These results can be explained by the schema in Fig. 9C as similar to the inhibitory effect of SHP-2-(198–593). SHP-2-(198–593) reveals a significantly high level of SHP-2 phosphatase activation (Fig. 3B). Since PDGFB receptor can be a substrate of SHP-2 tyrosine phosphatase, it is possible that SHP-2-(198–593) dephosphorylates tyrosine-phosphorylated PDGFB receptor, thereby preventing PDGF-induced Elk-1 activation. Further investigation is needed to clarify the inhibitory mechanism of SHP-2-(198–593).

Maroun et al. (16) reported that the expression of a catalytically inactive SHP-2 protein (SHP-2C/S) abrogates Met-induced ERK activation, suggesting that SHP-2 phosphatase activity is required for Met receptor-induced ERK1/2 activation. These results can be explained by the schema in Fig. 9B. Of note, we found that SHP-2-(399–593) activated counterpart SHP-2 phosphatase activity, but SHP-2-(399–593C/S) inhibited PDGF-induced SHP-2 phosphatase activity. Since both mutants of SHP-2-(399–593) and SHP-2-(399–593C/S) do not have phosphatase activity, the inhibition of wild type SHP-2 phosphatase activity mediated by SHP-2-(399–593C/S) may be due to the conformation change by the binding of SHP-2 mutants, which are mutated at Cys429 to Ser. Further investiga-
tion is needed to clarify the inhibitory mechanism for SHP-2-(399–593C/S).

As we stated in the Introduction, there are three possible models to explain the role of SHP-2 in Elk-1 activation: 1) the combination of both SHP-2-Gab1 complex formation and SHP-2 phosphatase activity is critical for ERK1/2 and Elk-1 activation; 2) Gab1 associates with N-terminal SH2 domains of SHP-2, increases SHP-2 phosphatase activity, and then activates ERK1/2 and Elk-1 activity; or 3) the association of Gab1 with SHP-2 is the only critical mechanism to regulate ERK1/2 and Elk-1 activity, and SHP-2 phosphatase activity is not directly involved in this process. Model 2 can explain the first
step of SHP-2 activation in Fig. 9A but not the inhibitory effect of SHP-2-(198–593), which is a constitutively active form of SHP-2, on Elk-1 activation (Fig. 9C). In contrast, it is possible to explain the inhibitory effect of SHP-2-(198–593) (Fig. 9C) on Elk-1 activation by model 3. However, the inhibitory effect of SHP-2C/S and SHP-2-(399–593C/S) cannot be explained by model 3. If “trapping” of Gab1 by SHP-2C/S is the main inhibitory mechanism of SHP-2C/S as explained in the Introduction, it is difficult to explain how SHP-2-(399–593) can activate Elk-1, because SHP-2-(399–593) has no phosphatase activity but can associate with Gab1 similarly to SHP-2C/S. Therefore, model 1 is the most likely mechanism of SHP-2 function in Elk-1 activation shown in Fig. 9A.

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REFERENCES

1. Feng, G. S. (1999) Exp. Cell Res. 253, 47–54
2. Wong, L., and Johnson, G. R. (1996) J. Biol. Chem. 271, 20961–20964
3. Qi, J. H., Ito, N., and Claesson-Welsh, L. (1999) J. Biol. Chem. 274, 14455–14463
4. Shi, Z. Q., Lu, W., and Feng, G. S. (1998) J. Biol. Chem. 273, 4904–4908
5. Heldin, C. H., Ostman, A., and Ronnstrand, L. (1998) Biochim. Biophys. Acta 1378, 79–113
6. Hackel, P. O., Zwick, E., Prenzel, N., and Ulrich, A. (1999) Curr. Opin. Cell Biol. 11, 184–189
7. Zhao, Z., Larroque, R., He, W. T., Fischer, E. H., and Shen, S. H. (1994) J. Biol. Chem. 269, 8780–8785
8. Dechert, U., Adam, M., Harder, K. W., Clark-Lewis, I., and Djurj, F. (1994) J. Biol. Chem. 269, 5602–5611
9. Hof, P., Plaskey, S., Dhe-Paganon, S., Eek, M. J., and Shoelson, S. E. (1998) Cell 92, 441–450
10. Xu, F., Zhao, R., Peng, Y., Guerra, A., and Zhao, Z. J. (2001) J. Biol. Chem. 276, 29479–29484
11. Oh, E. S., Gu, H., Sayton, T. M., Timms, J. F., Hausdorff, S., Frevert, E. U., Kahn, B. B., Pawson, T., Neel, B. G., and Thomas, S. M. (1999) Mol. Cell. Biol. 19, 3205–3215
12. Guat, P., Giordano, S., Williams, T. A., Roetti, S., Van Obberggen, E., and Comoglio, P. M. (2000) Oncogene 19, 1509–1518
13. Hayashi, H., Ichihara, M., Iwashita, T., Murakami, H., Shimoto, Y., Kawai, K., Kurakawa, K., Murakumo, Y., Imai, T., Funahashi, H., Nakao, A., and Takahashi, M. (2000) Oncogene 19, 4469–4475
14. Lamorte, L., Kamikura, D. M., and Park, M. (2000) Oncogene 19, 5973–5981
15. Rakshit, S., Pyne, S., and Pyne, N. J. (2000) Mol. Pharmacol. 58, 413–420
16. Maroun, C. R., Naujokas, M. A., Belartado-Madurga, M., Weng, A. J., and Park, M. (2000) Mol. Cell. Biol. 20, 8535–8525
17. Cunnick, J. M., Mei, L., Doupnik, C. A., and Wu, J. (2001) J. Biol. Chem. 276, 24380–24387
18. Che, W., Abe, J., Yoshizumi, M., Huang, Q., Glassman, M., Ohta, S., Melaragno, M. G., Poppa, V., Yan, C., Lerner-Marmarosh, N., Zhang, C., Wu, Y., Arlinghaus, R., and Berk, B. C. (2001) Circulation 104, 1399–1406
19. Saxton, T. M., Henke, M., Wasco, S. S., Chen, R., Uhl, C. J., Skalab, F., Feng, G. S., and Pawson, T. (1997) EMBO J. 16, 2352–2364
20. Yoshizumi, M., Abe, J., Haemeler, J., Huang, Q., and Berk, B. C. (2000) J. Biol. Chem. 275, 11706–11712
21. Tang, H., Zhao, Z. J., Huang, Y. X., Landos, E. J., and Inagami, T. (1999) J. Biol. Chem. 274, 12401–12407
22. Yau, J., Lu, H., Lee, J. M., Abe, J., and Berk, B. C. (2001) J. Biol. Chem. 276, 10870–10874
23. Shi, Z. Q., Yu, D. H., Park, M., Marshall, M., and Peng, G. S. (2000) Mol. Cell. Biol. 20, 1526–1536
24. Cunnick, J. M., Doupnik, C. A., and Wu, J. (2000) J. Biol. Chem. 275, 13842–13848