The Src Homology 2 (SH2) Domain of SH2-containing Inositol Phosphatase (SHIP) Is Essential for Tyrosine Phosphorylation of SHIP, Its Association with Shc, and Its Induction of Apoptosis*

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Ling Liu‡, Jacqueline E. Damen‡, Michael R. Hughes§, Ivan Babic, Frank R. Jirik, and Gerald Krystal¶

From the Terry Fox Laboratory, British Columbia Cancer Agency, and The Biomedical Research Centre, University of British Columbia, Vancouver, British Columbia V5Z 1L3, Canada

In this study we have investigated the role that the Src homology 2 domain (SH2) of the 145-kDa 5-phosphatase, SH2-containing inositol phosphatase (SHIP), plays in three of the properties that have been associated with this protein following cytokine stimulation: its association with Shc, its tyrosine phosphorylation, and its inhibition of hemopoietic cell growth. In vitro studies using this SH2 domain revealed that it was capable of binding directly to the Tyr(P)317 motif of Shc with a KD of approximately 290 nM, in keeping with other specific SH2/Tyr(P) interactions. In vitro analysis revealed the SH2 domain of SHIP to act together with the Tyr(P)317 and phosphotyrosine binding (PTB) domains of Shc, respectively, to ensure a high affinity SHIP-Shc complex. Expression of cDNAs encoding hemagglutinin-tagged wild type and SH2-inactivated forms of SHIP in the murine hemopoietic cell line DA-ER revealed that wild type SHIP becomes both tyrosine-phosphorylated and associated with Shc following interleukin-3 stimulation, as expected, but the SH2-inactivated SHIPs do neither. Moreover, while the growth rates of parental DA-ER cells and cells expressing these various SHIP constructs are identical, the wild type SHIP-expressing cells die, via programmed cell death, far more rapidly than parental cells. Cells expressing SH2-inactivated SHIPs, on the other hand, show either a reduced or no effect on apoptosis. These results suggest that the SH2 domain of SHIP is required not only for the tyrosine phosphorylation of SHIP and Shc association following cytokine stimulation but also for its induction of apoptosis.

Several years ago we (1, 2) and others (3, 4) identified a 145-kDa protein (p145) within hemopoietic cell lines that became tyrosine-phosphorylated and associated with Shc following stimulation with various cytokines. Interestingly, we found that the association between p145 and Shc could be inhibited with phosphopeptides corresponding to the pY317VNV sequence within Shc (2). Based on these results we proposed that p145 contained a Src homology 2 domain (SH2)2, and that it competed with Grb2 for binding to the Tyr(P)317 sequence within Shc (2). More recently we cloned the cDNA for this 145-kDa protein (5) and found that the predicted amino acid sequence indeed did contain an SH2 domain at its amino terminus as well as two phosphotyrosine binding (PTB) consensus sequences (i.e. NPYX sequences) (6), several proline rich SH3 binding regions, and two motifs highly conserved among inositol polyphosphate 5-phosphatases. Based on these properties we called this protein SHIP for SH2-containing inositol phosphatase (5). Unlike most inositol polyphosphate 5-phosphatases that hydrolyze phosphatidylinositol 4,5-P2-bisphosphate (PI-4,5-P2) and/or inositol 1,4,5-trisphosphate (7), SHIP selectively hydrolyzes the 5’-phosphate from inositol 1,3,4,5-tetraphosphate, and phosphatidylinositol 3,4,5-trisphosphate (PI-3,4,5-P3) (5), two inositol polyphosphates recently implicated in growth factor-mediated signaling (8–11).

Concurrent with our cloning of SHIP, Lioubin et al. (12) independently obtained the cDNA for this protein using a yeast two-hybrid system based on its affinity for the PTB domain of Shc. They further went on to show that ectopic expression of SHIP in FDC-P1 cells expressing the macrophage colony-stimulating factor receptor, c-Fms, led to a reduction in the size of the macrophage colony-stimulating factor receptor or interleukin-3 (IL-3)-stimulated colonies in soft agar (12). Subsequently, Kavanaugh et al. monitored the purification of this 145-kDa protein by its ability to bind to the Shc PTB in vitro. Their strategies indicated that Shc was capable of binding via its PTB domain to SHIP, at least in vitro, and this was potentially at odds with our finding that phosphopeptides corresponding to the pY317VNV motif within Shc inhibit SHIP binding to Shc (2). Because of the potential importance of the interaction of SHIP with Shc in mediating cytokine-induced signals we were interested in establishing whether the SH2 domain of SHIP played a role in this interaction as well as in its reported inhibitory effect on cell growth.

EXPERIMENTAL PROCEDURES

Reagents—The production and purification of COS cell-derived murine IL-3 and granulocyte macrophage colony-stimulating factor were as described previously (14). Glutathione S-transferase (GST) fusion * This work was supported by the National Cancer Institute of Canada and the Medical Research Council of Canada with core support from the British Columbia Cancer Foundation and the British Columbia Cancer Agency. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ The first two authors contributed equally to this work.
§ Holds an NSERC studentship.
¶ A Terry Fox Cancer Research Scientist of the National Cancer Institute of Canada supported by funds from the Canadian Cancer Society. To whom correspondence should be addressed: Terry Fox Laboratory, British Columbia Cancer Research Centre, 601 West 10th Ave., Vancouver, British Columbia V5Z 1L3, Canada. Tel.: 604-877-6070; Fax: 604-877-0712.

1 The abbreviations used are: SH2, Src homology 2; pY, phosphotyrosine; FACS, fluorescence-activated cell sorter; GST, glutathione S-transferase; HA, hemagglutinin; IL-3, interleukin-3; mAb, monoclonal antibody; PTB, phosphotyrosine binding; FLC, phospholipase C; PI, phosphatidylinositol; SHP, SH2-containing inositol phosphatase; WT-SHIP, wild type SHIP; ΔSH2-SHIP, SHIP lacking the second half of the SH2 domain; R34G-SHIP, SHIP with a critical arginine in the SH2 domain replaced with a glycine.
proteins consisting of the 27-kDa amino terminus of GST linked to the SH2 domain of murine SHIP (amino acids 7–133) (5), bovine PLC-γ1 (amino-terminal) (residues 547–659), and murine Grb2 (residues 60–158) were expressed in *Escherichia coli* in pGEX-2T plasmids (Pharmacia Biotech Inc., Baie d’Urfe, Quebec, Canada) and purified from the sonicated bacteria using glutathione-agarose (Pharmacia) as described previously (1). Rabbit antiserum to SHIP was generated by immunizing animals with the GST-SHIP SH2 fusion protein described above. The phosphopeptides used for the inhibition studies consisted of the 12-mer RRSVSPVYVNQVNL corresponding to the sequence flanking Tyr<sup>317</sup> within Shc, the 11-mer EMINPNpYIGMG corresponding to the sequence flanking the NPXY within SHIP (both synthesized by the Sequencing Center at the University of Victoria, British Columbia, Canada), and the 11-mer STDpYSSGGSQG corresponding to an intracellular region of the erythropoietin receptor (15) (generously provided by Dr. Taolin Yi, Cleveland Clinic Foundation, Cleveland, OH). The anti-phosphotyrosine (anti-Tyr(P)) monoclonal antibody (mAb) was purchased from Upstate Biotechnology (Lake Placid, NY). Both affinity-purified rabbit polyclonal antibodies to Shc (for immunoprecipitations) and mAb to Shc (for Western blotting) were obtained from Transduction Laboratories (Lexington, KY). The anti-hemagglutinin (HA) mAb was from Babco (Richmond, CA). Horseradish peroxidase-conjugated second antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Protein grade Nonidet P-40 was from Calbiochem. The enhanced chemiluminescence Western blotting reagents were obtained from Pierce.

**Generation of DA-ER Cells Expressing Hemagglutinin-tagged Forms of SHIP**—HA-tagged forms of wild type (WT)-SHIP, ΔSH2-SHIP (lacking the second half of the SH2 domain), and R34G-SHIP (in which the critical arginine in the FLVR sequence of the SH2 domain has been replaced with a glycine (9)) were generated from first fusing the HA-tag in-frame at the 5′-end to the entire murine WT-SHIP cDNA in a murine stem cell virus vector containing the puromycin resistance gene, *pac* (16). The ΔSH2-SHIP was then generated from this construct by deleting the *Fsp*I, AccI fragment corresponding to amino acids 44–149 within the SH2 domain. The R34G point mutant was generated from the HA-tagged WT-SHIP using the QuikChang<sup>™</sup> site-directed mutagenesis kit (Stratagene). All three plasmids were calcium phosphate- transfected into the producer cell line BOSC 2t, and 48-h retroviral supernatants were used to infect DA-ER cells. DA-ER cells were selected for 2 weeks in puromycin, and clones were analyzed for SHIP expression by Western analysis with anti-HA mAbs.

**Immunoprecipitations and Western Blotting**—Murine B6SUtA1 cells, maintained in RPMI 1640 medium with 10% fetal calf serum and 5 ng/ml granulocyte-macrophage colony-stimulating factor or murine DA-ER cells (DA-3 cells expressing cell surface erythropoietin receptors (1)) infected with HA-tagged WT-, R34G-, or ΔSH2-SHIP, maintained in RPMI 1640 medium with 10% fetal calf serum and 5 ng/ml IL-3, were growth factor-deprived for 4–6 h at 37 °C in RPMI 1640 medium containing 0.1% bovine serum albumin and then stimulated at 37 °C for 5 min with murine IL-3 (400 ng/ml). The cells were then washed once with phosphate-buffered saline, solubilized at 2×10<sup>6</sup> cells/ml with 0.5% Nonidet P-40 in 4 °C phosphorylation solubilization buffer (50 mM HEPES, pH 7.4, 100 mM NaF, 10 mM Na<sub>3</sub>PO<sub>4</sub>, 2 mM Na<sub>2</sub>VO<sub>4</sub>, 4 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 2 μg/ml aprotinin), and subjected to immunoprecipitation and Western blotting as described previously (2).

**In Vitro Binding Assays**—Cell lysates from B6SUtA1 cells, treated with or without IL-3 for 5 min at 37 °C, were either incubated immediately for 1 h at 4 °C with glutathione-agarose beads bearing a GST-fusion protein containing the SH2 domain of SHIP (lanes 1 and 2) or boiled first in SDS, diluted with Nonidet P-40 and then incubated with these beads (lanes 3 and 4). Bead-bound material was then subjected to Western analysis with anti-Tyr(P) (α-PY) mAbs. The lower panel is a reprobing of this blot with anti-Shc antibodies. B, the relative binding of the GST-SH2 fusion proteins of SHIP, Grb2, and the amino-terminal SH2 of PLC-γ1 for the Tyr<P><sup>317</sup> phosphopeptide of Shc is shown when applied to the same binding surface at 1 μM concentration. Equilibrium data from the binding analysis at several protein concentrations were used to calculate affinities. None of the fusion proteins bound to the dextran matrix in the absence of cross-linked Tyr<P><sup>317</sup> phosphopeptide. buffer and subjected to Western analysis with the anti-Tyr(P) mAb, 4G10.

SH2 Binding Studies Using Surface Plasmon Resonance—GST fusion proteins containing the SH2 domains of Grb2, SHIP, and the amino-terminal SH2 of bovine PLC-γ1 were eluted from glutathione-agarose beads at 23 °C with 75 mM HEPES, 150 mM NaCl, 5 mM dithiothreitol, 0.1% Triton X-100, and 20 mM reduced glutathione and dialyzed for 2 days in phosphate-buffered saline, and protein concentrations were determined using the bicinchoninic acid protein assay reagent (Pierce). The binding affinities of the GST-SH2 fusion proteins were measured with a BLACore biosensor (18). The 12-mer phosphopeptide corresponding to the sequence flanking Tyr<sup>317</sup> within Shc was
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Apoptosis Assays—For DNA laddering studies, 2 × 10⁶ DA-ER cells expressing HA-tagged WT- and mutant SHIPS were lysed in 50 mM of 50 mM Tris-HCl, pH 7.5, and 20 mM EDTA containing 0.5% Nonidet P-40, and protein and RNA were removed by digestion with RNase A (5 μg/μl) and proteinase K (2.5 μg/μl), respectively, and the ethanol-precipitated DNA was dissolved in gel-loading buffer and electrophoresed using 2% agarose gels containing 0.1 μg/ml EtBr (19). For propidium iodide and fluorescence-activated cell sorter analysis (FACS), 1 × 10⁶ DA-ER cells expressing HA-tagged WT- and mutant SHIPS were washed in phosphate-buffered saline, incubated for 5 min in the dark in 1 ml of 50 mM sodium citrate, pH 8.0, 20 μg/ml propidium iodide, and 0.1% Triton X-100, and analyzed by FACS (20).

RESULTS AND DISCUSSION

The SH2 Domain of SHIP Binds Directly to Shc in Vitro—To confirm our earlier studies, which suggested that p145 (SHIP) interacted via its SH2 domain with Shc (2), lysates from B6SUtA1 cells, treated with and without IL-3, were incubated with a bead-bound GST-fusion protein containing the SH2 domain of SHIP, and the bound proteins were subjected to Western analysis using anti-Tyr(P) mAbs. As can be seen in the first two lanes of Fig. 1A, the SH2 domain of SHIP bound both a 70- and a 56-kDa tyrosine-phosphorylated protein following IL-3 stimulation. To determine whether these phosphorylated proteins bound directly or indirectly to this SH2 domain, lysates were boiled in SDS before dilution and incubation with the beads (Fig. 1A, lanes 3 and 4). This resulted in the disappearance of the 70-kDa protein (which reprobing experiments demonstrated to be the tyrosine phosphatase, SHP2 (also known as Sypl)) but not the 56-kDa protein. Reprobing this blot with anti-Shc antibodies demonstrated that the 56-kDa protein was Shc (Fig. 1A, lower panel) and that the SH2 domain of SHIP binds directly to Shc in vitro.

The SH2 Domain of SHIP Has an Affinity for the Tyr(P)³¹⁷ Motif of Shc That Is Consistent with Other SH2/Tyr(P) Interactions—To determine if the SH2 domain of SHIP was capable of binding to the Tyr(P)³¹⁷ motif of Shc and also to gain some insight into the affinity of the SH2 domain of SHIP for this motif, we examined the relative binding affinities of GST-fusion proteins containing the SH2 domains of SHIP, Grb2, and PLC-γ1 for the Shc Tyr(P)³¹⁷ phosphopeptide using surface plasmon resonance. From three representative experiments, each with a different concentration of the cross-linked phosphopeptide, titration curves were carried out with the SH2 domains of Grb2 and SHIP, and the apparent Kₐ values calculated from equilibrium data were determined to be approximately (2.9 ± 1.3) × 10⁻⁶ M and (2.8 ± 0.3) × 10⁻⁵ M for the SHIP-SH2 and Grb2-SH2 fusion proteins, respectively. The PLC-γ1 amino-terminal SH2 fusion did not show any affinity for the binding surface up to a GST-SH2 concentration of 10 μM. Fig. 1B depicts the curves generated at 1 μM concentration of each SH2 domain for comparative purposes. This confirms that the SH2 domain of SHIP is capable of specifically interacting with the Tyr(P)³¹⁷ motif of Shc and that the affinity of this interaction is consistent with that reported for other SH2

![Figure](https://example.com/figure1.png)

**Figure 2.** The association of SHIP with Shc is mediated in vitro by both SH2/Tyr(P)³¹⁷ and NPNpY/PTB interactions. Lysates from B6SUtA1 cells, treated with and without IL-3, were immunoprecipitated with anti-Shc antibodies in the presence and absence of phosphopeptides corresponding to the NPNpY and Tyr(P)³¹⁷ motifs or a control phosphopeptide, and the precipitates were subjected to Western analysis with anti-Shc antibodies. The lower panel is a reprobing of this blot with anti-ShIP antibodies to demonstrate equal loading.

**Figure 3.** SHIP mutants lacking a functional SH2 domain do not bind Shc and do not become tyrosine-phosphorylated in response to IL-3 stimulation. A, Western analysis using anti-HA antibodies of total cell lysates from IL-3-stimulated DA-ER cells expressing HA-tagged WT-, ΔSH2-, and R34G-SHIP. B, immunoprecipitates of these same lysates with anti-Shc antibodies prior to Western analysis with anti-HA antibodies. Reprobing this blot with anti-Shc antibodies demonstrated equal loading (data not shown). C, these same cell lysates were subjected to anti-Tyr(P) immunoprecipitation and Western analysis with anti-HA mAbs.
Both the SH2 and NPxY Domains of SHIP Participate in the Association of SHIP with Shc in Vivo—Lioubin et al. (12) and Kavanaugh et al. (13) recently demonstrated that the PTB domain of Shc is capable of binding to SHIP via the NPxY motifs of SHIP in vitro (and we have confirmed this using bead-bound GST fusion proteins containing the PTB domain of SHIP, data not shown). To determine whether the SHIP/Shc association in vivo can be mediated solely via SH2/Tyr(P)317 or NPxY/PTB interactions, lysates from B6SUtA1 cells, treated with and without IL-3 for 5 min, were subjected to anti-SHIP immunoprecipitation in the presence and absence of phosphopeptides corresponding to the NPxY and Tyr(P)317 sequences in SHIP and Shc, respectively. Western analysis, using anti-Shc mAbs revealed that both phosphopeptides, but not a control phosphopeptide, inhibited the co-precipitation of Shc with SHIP (Fig. 2, top panel). Probing this blot with anti-Tyr(P) mAbs demonstrated that the tyrosine phosphorylation level of SHIP was unaffected by the competing phosphopeptides, suggesting that these phosphopeptides were not causing SHIP/Shc dissociation by binding to and activating a SHIP or Shc-associated SH2-containing tyrosine phosphatase (data not shown). Reprobing this blot with anti-SHIP antibodies confirmed equal loading (lower panel). Since both the NPxY and Tyr(P)317 phosphopeptides were capable of disrupting SHIP/Shc complexes it suggests that the SH2 domain and the NPxY motif of SHIP are both required for a high affinity interaction with Shc. This dual interaction may explain, in part, why we observed substantial levels of both Shc-SHIP and Shc-Grb2 complexes in these cells even though our BIAcore results indicated that the isolated SH2 domain of SHIP had a 10-fold lower affinity than the isolated SH2 domain of Grb2 for the Tyr(P)317 motif of Shc. In this regard it should be pointed out that anti-Grb2 immunoprecipitates do not contain SHIP (2), and anti-SHIP immunoprecipitates do not contain Grb2 in B6SUtA1 cells (data not shown). Thus SHIP and Grb2 may compete in vivo for Shc.

SHIP Mutants Lacking a Functional SH2 Domain Do Not Bind Shc and Do Not Become Tyrosine Phosphorylated in Vivo in Response to IL-3—To explore the relevance of this SH2 domain to downstream events in vivo, DA-ER cells were retrovirally infected with cDNAs for 5’-HA-tagged forms of WT-, ΔSH2,-lacking a functional SH2 domain), and R34G- (lacking a critical arginine in the FLVR sequence of the SH2 domain) (9) SHIP. The constructs were shown to retain the proper coding frame by sequencing the resulting cDNA and demonstrating that the protein products were immunoprecipitable with anti-15-mer anti-SHIP antiserum (5). Moreover, anti-HA antibody immunoprecipitates from these infected cells, but not uninfected cells, possessed SHIP inositol polyphosphate 5-phosphatase activity, and the specific activity (i.e. inositol polyphosphate 5-phosphatase activity/mg of SHIP) was similar in all constructs (data not shown). DA-ER cell clones expressing HA-WT-SHIP, HA-ΔSH2-SHIP, and HA-R34G-SHIP were then stimulated with IL-3, and total cell lysates were subjected to Western analysis with anti-HA antibodies (Fig. 3A). This revealed that while all three constructs gave rise to lower molecular weight forms of SHIP, most likely through proteolytic degradation at the carboxyl terminus (since they were HA-tagged at the amino terminus), they clearly expressed the expected full-length forms. Aliquots of these same cell lysates and two clones each of HA-tagged WT-, ΔSH2-, and R34G-SHIP, taken on day 2. Lane 1, parental DA-ER; lanes 2 and 3, WT-SHIP6 and WT-SHIP12, respectively; lanes 4 and 5, two clones of ΔSH2-SHIP; lanes 6 and 7, two clones of R34G-SHIP.
were also immunoprecipitated with anti-Shc antibodies prior to Western analysis with anti-HA antibodies. This revealed that HA-WT-SHIP co-precipitated with Shc, as expected, but HA-ΔSH2-SHIP and HA-R34G-SHIP did not (Fig. 3B). The absence of the lower molecular weight forms of HA-WT-SHIP in this anti-Shc immunoprecipitate most likely reflects the requirement of the carboxyl-terminal NPXY motifs of SHIP for the in vivo association of SHIP with Shc.

The same DA-ER cell clones were treated with and without IL-3, and lysates were subjected to anti-Tyr(P) mAbs immunoprecipitation and Western analysis with anti-HA mAbs. As can be seen in Fig. 3C, the HA-ΔSH2-SHIP and HA-R34G-SHIP were not detectably tyrosine-phosphorylated, while HA-WT-SHIP was strongly phosphorylated under the same conditions. The reciprocal experiment, in which anti-HA immunoprecipitates were carried out and Western analysis performed with anti-Tyr(P) mAbs, yielded identical results (data not shown). Thus the SH2 domain of SHIP is also required for SHIP to become tyrosine phosphorylated in response to IL-3 stimulation. Interestingly, this effect on its tyrosine phosphorylation suggests that the SH2 domain of SHIP may play a role either in the binding of SHIP to a tyrosine kinase (directly or through its interaction with Shc) or in the translocation of SHIP to a tyrosine kinase. Related to this, it has been reported that IL-3 stimulates not only the activation of the IL-3R-associated tyrosine kinase Jak2 (22) but also Fps/Fes (23) and various Src family members (24, 25).

**SHIP Reduces the Viability of Confluent DA-ER Cells and Its SH2 Domain Is Required for This Effect**—In preliminary studies to investigate the biological role of SHIP we found we could not express HA-WT-SHIP in DA-ER cells at more than twice endogenous SHIP levels, perhaps reflecting a reduced ability of these cells to survive. Lioubin et al. (12) also found they could not express exogenous WT-SHIP at more than twice endogenous levels in FD-Fms cells. Nonetheless, even at these slightly elevated levels, they observed an inhibition of the macrophage colony-stimulating factor receptor-induced colony size (12). To gain some insight into the biological consequences of overexpressing WT SHIP in DA-ER cells, suspension cultures containing parental DA-ER and two independently isolated clones infected with HA-tagged WT-SHIP were initiated with 2 × 10^5 cells/ml in 10% fetal calf serum plus 5 ng/ml IL-3, and duplicate samples were counted following trypan blue-staining every 24 h for 4 days. As can be seen in Fig. 4A, the exponential growth rate of all the cells was identical. However, once confluence was achieved the survival of the two cell types was markedly different. Interestingly, cell counts at various times during days 2 and 3 revealed that the total number of cells (i.e., the trypan blue positive plus negative cells) was actually the same in all the cultures, but the level of trypan blue positive cells was substantially higher in the WT SHIP cultures. These results suggested that overexpression of WT SHIP reduced the viability of confluent DA-ER cells. This effect was not observed with logographically growing DA-ER cells starved of growth factor.

We then compared the viability of two independently isolated clones of HA-WT-SHIP, HA-ΔSH2-SHIP, and HA-R34G-SHIP expressing DA-ER cells with parental cells and found that the two HA-ΔSH2-SHIP-infected clones displayed a viability pattern in between that seen with parental and WT cells while the HA-R34G-SHIP-infected clones consistently possessed a viability equal to or slightly greater than parental cells (Fig. 4B). To determine if the WT-SHIP-induced loss of viability was mediated by an apoptotic pathway, DNA was extracted from the different cell clones before differences in viability were detected by trypan blue exclusion, i.e., on day 2, and DNA fragmentation was evaluated by electromorphs on agarose gels (19). As can be seen in Fig. 4C, the extent of DNA laddering was far greater in the two clones expressing HA-WT-SHIP than in the parental or HA-R34G-SHIP cell clones. The two HA-ΔSH2-SHIP clones, on the other hand, showed an intermediate pattern (consistent with our trypan blue studies). DNA fragmentation was also assessed on days 2 and 3 by FACS using propidium iodide staining (20), and the results were consistent with our trypan blue staining and our DNA laddering results. For example, on day 2, HA-WT-SHIP-expressing cells showed the most DNA fragmentation (e.g. 22 ± 0.5%), HA-ΔSH2-SHIP-expressing cells showed less (15 ± 3.8%), and parental (2-3 ± 0.5%) and R34G-SHIP (3.5 ± 0.1%) expressing cells had the least. The SH2 domain of SHIP thus appears to be critical for the apoptotic effect of SHIP since a very subtle single amino acid change (R34G) in full-length SHIP, in an amino acid which has been shown previously to be critical for the binding of SH2 domains to tyrosine-phosphorylated residues (9), completely eliminates this effect. Interestingly, a complete elimination of this SHIP effect was not observed with cells expressing the HA-ΔSH2-SHIP. This may be due to the fact that a large section of the SH2 domain was removed to generate this construct, and this may have resulted in a significant conformational change and subsequent unpredictable effects on signaling.

Mechanistically, this SHIP-induced loss of viability could occur through the ability of SHIP to compete with Grb2 for Shc and thus, potentially, reduce Ras activation. This is consistent with a recent report by Kinoshita et al. (26), in which they showed that the Ras pathway plays an important role in preventing apoptosis in IL-3-stimulated cells. Alternatively, since PI 3-kinase has been shown to prevent apoptosis in certain cell types (27), SHIP might reduce cell viability by hydrolyzing the primary in vivo product of PI 3-kinase, PI 3,4,5-P_3.

We are currently generating inducible SHIP vectors so that the effects of WT and mutant forms of SHIP on intracellular signaling and various biological end points can be further investigated.

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