Src family tyrosine kinases regulate adhesion-dependent tyrosine phosphorylation of 5′-inositol phosphatase SHIP2 during cell attachment and spreading on collagen I

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Summary
Inositol phosphatases play an important role in regulation of cellular levels of lipid second messengers. Recently we have reported a novel function for SHIP2 in cell adhesion and spreading. In this study, we further characterize the adhesion-dependent tyrosine phosphorylation of SHIP2 and examine the role of Src family tyrosine kinases in the regulation of SHIP2 function. SHIP2 was tyrosine phosphorylated during cell attachment and spreading on collagen I, but not on fibronectin, collagen IV, laminin or poly-L-lysine. SHIP2 tyrosine phosphorylation, induced by plating on a collagen-I-coated surface but not by epidermal growth factor or insulin treatment of cells, was completely blocked by small molecule inhibitors of Src family kinases. SHIP2 could be phosphorylated in vitro by recombinant Src kinase and tyrosines 986-987 in the NPXY motif of SHIP2 appear to be the major sites of phosphorylation for Src both in vitro and in vivo. An activated form of Src induced strong tyrosine phosphorylation of SHIP2 while a dominant-negative form decreased collagen-I-dependent SHIP2 phosphorylation. SHIP2 associated with the adapter protein Shc via its NPXY motif during cell spreading on collagen I in a Src activity-dependent manner. Expression of SHIP2 with mutated NPXY motif caused deregulation of lamellipodia formation during spreading on collagen I. These observations indicate that SHIP2 is regulated by Src family kinases during cell attachment and spreading on collagen I and suggest an important role for SHIP2 as a part of a signaling pathway that regulates actin cytoskeleton remodeling.

Key words: 5′-inositol phosphatase, SHIP2, Src kinase, Shc, Adhesion, Collagen I

Introduction
Metabolites of phosphatidylinositol (PtdIns) serve as important second messengers that regulate cellular signaling pathways. Among several cellular enzymes that specifically modify PtdIns, phosphoinositide 3-kinase (PI 3-kinase) is shown to be pivotal in growth factor, insulin and G protein-mediated signal transduction (Corvera and Czech, 1998). In addition, PI 3-kinase is implicated in the regulation of adhesion and migration (Jones et al., 2000). Specific inhibitors of PI 3-kinase inhibited adhesion and migration in a variety of cell types (Ji and Haimovich, 1999; King et al., 1997; Zheng et al., 2000). Activated PI 3-kinase localizes to cell-cell as well as cell-matrix adhesion sites in epithelial cells and to membrane ruffles in fibroblasts (Watton and Downward, 1999). Activation of Akt/PKB, a downstream target of the PI 3-kinase pathway occurs upon integrin ligation (Khwaja et al., 1997; King et al., 1997). The regulatory p85 subunit of PI 3-kinase interacts with proteins regulating adhesion and migration such as focal adhesion kinase (FAK), Src and p130Crk-associated substrate (p130Cas) (Bachelot et al., 1996; Fukui and Hanafusa, 1989; Li et al., 2000). Recruitment and activation of Src to the integrin-extracellular matrix (ECM) contacts occurs early in the adhesion process (Fincham et al., 1996). A number of these Src substrates, including p130Cas, paxillin, cortactin and talin, play a critical role in actin cytoskeleton rearrangements, cell spreading and migration (Jones et al., 2000; Schwartz, 2001).

Inositol phosphatases regulate the cellular levels of lipid second messengers (Majerus et al., 1999). A 3′-inositol phosphatase PH-TEN/MMAC1 is frequently inactivated in tumor cells leading to increased PI 3-kinase product, phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃] resulting in activation of Akt/PKB. PTEN also regulates integrin-mediated activation of extracellular signal regulated kinase (ERK), interacts with FAK and inhibits adhesion, migration and invasion processes (Di Cristofano and Pandolfi, 2000). The 5′-inositol phosphatases, SH2-containing inositol 5-phosphatase 1 and 2 (SHIP1 and SHIP2) specifically dephosphorylate PtdIns(3,4,5)P₃, and inositol (1,3,4,5)-tetrakiphosphate [Ins(1,3,4,5)P₄] on the D5 position of the inositol ring (Erneux et al., 1998). While SHIP1 expression is restricted primarily to hematopoietic tissues, SHIP2 appears to be ubiquitous (Habib et al., 1998; Rohrschneider et al., 2000). Analogous to the negative regulation of growth factor and antigen receptor-mediated signaling by SHIP1, some studies suggest that SHIP2 downregulates insulin and FcγRIIB receptor signaling (Muraille et al., 1999; Wada et al., 2001).
Targeted deletion of SHIP2 in mice produced neonatal fatality attributed to hypoglycemia and insulin hypersensitivity (Clement et al., 2001). Overexpressed SHIP2 downregulated Akt activation and caused cell-cycle arrest (Taylor et al., 2000). In addition, the same group reported that SHIP2 effectively utilizes phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P2] as its substrate in addition to already reported PtdIns(3,4,5)P3 and Ins(1,3,4,5)P4 (Taylor et al., 2000).

Besides an N-terminal SH2 domain, both SHIP1 and SHIP2 possess proline-rich regions and NPXY motifs (two in SHIP1 and one in SHIP2) serving as potential protein–protein interaction sites (Rohrschneider et al., 2000). SHIP2 also has a C-terminal SAM domain that is not present in SHIP1. Recently, we reported that an important regulator of adhesion and migration processes, p130Cas, interacted with the SH2 domain of SHIP2 (Prasad et al., 2001). In HeLa cells, SHIP2 localized to focal contacts during attachment and to lamellipodia in spreading cells. Wild-type SHIP2 promoted adhesion while catalytically inactive SHIP2 inhibited spreading of HeLa cells. In this report, we further characterize the involvement of SHIP2 in adhesion providing evidence that SHIP2 tyrosine phosphorylation specifically occurs during cell attachment and spreading on collagen I, and that phosphorylation is mediated through activation of Src family kinases.

Materials and Methods

Materials

Anti-FLAG (M2) monoclonal antibody, a rabbit polyclonal anti-FLAG antibody, rat-tail collagen I, collagen IV, laminin, poly-L-lysine, phallolidin-TRITC were from Sigma Chemicals. Collagen I (rat tail) was also purchased from Roche Biochemicals. Anti-phosphotyrosine (clone 4G10) and anti-Src (clone GD11) antibodies were from Upstate Biotech. Monoclonal anti-Shc and rabbit polyclonal anti-Shc (clone 4G10) antibodies were from Upstate Biotech. Rabbit polyclonal anti-SHIP2 antisera was raised as described earlier (Habib et al., 1998). Expression vectors for activated Src and dominant-negative Src in pUSE.amp were purchased from Upstate Biotech.

Cell culture

HeLa, SH-SY5Y, Madin Darby canine kidney (MDCK) and 293T cells were routinely cultured in DMEM (with high glucose, pyridoxine hydrochloride, L-glutamine and without sodium pyruvate) containing 10% FBS. Culturing and induction of differentiation of 3T3-L1 adipocytes was done as described previously (Habib et al., 1998). Transient transfections of HeLa cells were carried out using Lipofectamine-Plus reagent (Invitrogen) according to the manufacturer’s instructions. Briefly, cells were cultured in 60 mm dishes 18-20 hours before transfection to obtain 30-40% confluence at the time of transfection. 2 μg DNA, 5 μl Plus reagent and 8 μl lipofectamine were used per 60 mm dish. Transfections were carried out for 5 hours at 37°C. Experiments were carried out 48 hours post-transfection. For cells cultured in 100 mm dishes, 7.5 μg DNA, 15 μl ‘Plus’ reagent and 25 μl lipofectamine were used. 293T cells were transfected by a modified CaPO4 method (Stratagene).

Construction of expression vectors encoding epitope-tagged SHIP2

cDNAs encoding full length SHIP2 with a FLAG epitope at the C-terminus (SHIP2-FLAG) were cloned into the pcDNA3 mammalian expression vector. Site-directed mutagenesis was used to replace the tyrosines with phenylalanines. These constructs were tagged with the FLAG epitope at the C-terminus.

Procedure for coating polystyrene (bacterial) petri dishes

Rat-tail collagen I was resuspended in 0.1 M acetic acid to 1 mg/ml concentration with stirring for 1-3 hours at room temperature (RT). Collagen I solution was stored at 4°C and pre-warmed to 37°C prior to coating. Non-tissue culture treated (bacterial) polystyrene plates were coated in phosphate buffered saline (PBS, 4.0 ml per dish) containing collagen I at indicated concentrations with lids open in the laminar-flow hood for 1 hour. Excess collagen solution was removed, washed twice with PBS and blocked in PBS containing 1% bovine serum albumin (BSA) in the hood with lids open for 30 minutes. Plates were washed twice more with PBS prior to usage. Coating of dishes with fibronectin (5 μg/cm²), collagen IV (6 μg/cm²), laminin (2 μg/cm²) and poly-L-lysine (0.01% solution, 0.5 ml/25 cm²) was carried out similarly.

Immunoprecipitation and western blot analyses

HeLa cells cultured under various conditions were processed as follows. For adherent (Ad) samples, confluent cells in 100 mm tissue dishes were serum starved for 3 hours in DMEM containing 0.5% BSA, washed once with cold PBS and scraped in HNTG lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.2 mM sodium orthovanadate, 1 mM PMSF and protease inhibitor cocktail from Boehringer Mannheim). Detached cell lysates (D) were prepared as follows. Cell monolayers were treated with 1× trypsin-EDTA (Invitrogen) for 3 minutes. Trypsin was inactivated by soybean trypsin inhibitor (1 mg/ml in DMEM containing 0.25% BSA). Cells were then centrifuged for 5 minutes at 50 g in a tabletop centrifuge and washed once with PBS prior to lysis in HNTG buffer. For samples from re-attaching cells, serum starved cells detached as above were re-plated in DMEM/0.5% BSA for indicated intervals on bacterial petri dishes coated with either collagen I or other attachment factors as indicated. At the indicated intervals, cells were gently washed once with cold PBS and adherent cells were scraped in HNTG lysis buffer. Medium and the PBS wash containing non-adherent cells (if any) were centrifuged and the resulting cell pellet was combined with the lysate from adherent cells. Immunoprecipitations from equal amounts of proteins and western blots were carried out as described previously (Prasad et al., 2000). For SHIP2-Shc co-immunoprecipitation experiments (shown in Fig. 8), NP-40 lysis buffer (20 mM Tris-HCl pH 7.5, 1% NP-40, 10% glycerol, 25 mM NaCl, 1 mM PMSF, 1 mM EDTA, 0.2 mM sodium orthovanadate and protease inhibitor cocktail from Boehringer Mannheim) was used. After immunoprecipitations with the indicated antibodies and protein A/G agarose, immune complexes were washed three times with NP-40 wash buffer (the same as NP-40 lysis buffer but containing 75 mM NaCl). Samples were then boiled with SDS-sample buffer prior to electrophoresis.

In vitro phosphorylation assays

FLAG-tagged wild-type SHIP2 or YY-FF (986-987) mutant proteins were expressed in 293T cells by transient transfection. 48 hours post-transfection, cells were lysed in HNTG buffer and FLAG-tagged proteins were purified using anti-FLAG (M2) antibody and protein A/G plus agarose. Purified wild-type and YY-FF mutant SHIP2 bound to protein A/G beads, were washed once with kinase assay buffer (10 mM Hepes pH 7.5, 10 mM MgCl2, 10 mM MnCl2, 1 mM DTT, 1 mM EDTA and 0.1 mM sodium orthovanadate) and resuspended in assay buffer. Beads were then incubated with 5 units of recombinant Src.
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**Fig. 1.** Collagen I induces SHIP2 tyrosine phosphorylation. Anti-SHIP2 or control pre-immune (Pre) immunoprecipitates (IP) from HeLa cells were blotted with anti-phosphotyrosine (α-PY). Anti-SHIP2 blots show amounts of SHIP2 protein in respective IP samples. The arrows point to tyrosine-phosphorylated SHIP2. (A) Time course: adherent (Ad), detached (D) or cells that were freshly plated on a collagen-I-coated (6 μg/cm²) surface (C.I) at indicated post-plating intervals were used for IP. (B) Coating density dependency: IPs were carried out as above. Cells were re-plated for 60 minutes on a polystyrene surface coated with increasing concentrations of collagen I (0.1 μg/cm² to 6 μg/cm²).

**Results**

**Cell attachment on collagen I induces SHIP2 tyrosine phosphorylation**

Previously we have observed adhesion-dependent tyrosine phosphorylation of SHIP2 in HeLa cells (Prasad et al., 2001). We wished to determine whether such a modification occurs in response to adhesion of cells to specific extracellular matrix proteins. When serum-starved HeLa cells were detached from tissue-culture-treated plastic by trypsinization, background levels of SHIP2 tyrosine phosphorylation were abolished as observed earlier. Re-plating of cells on a collagen-I-coated non-tissue-culture-treated polystyrene surface induced robust tyrosine phosphorylation of SHIP2 as observed by an anti-phosphotyrosine blot of anti-SHIP2 immunoprecipitates (Fig. 1A). SHIP2 phosphorylation appeared 30 minutes post-plating, was highest by 60 minutes and was persistent for up to 20 hours (data not shown). Two co-precipitating tyrosine-phosphorylated protein species (~180 kDa and 200-220 kDa) were apparent under such conditions. The identity of these SHIP2-associated proteins remains unknown. Our efforts to identify these proteins using specific antibodies against candidate proteins such as p190RhoGAP, talin, tensin and integrin β4 were unsuccessful (data not shown). The induction of SHIP2 tyrosine phosphorylation was detectable with a coating density of 0.5 μg/cm² and the maximum effect was seen with 3 μg/cm² (Fig. 1B). Plating on fibronectin, collagen IV or laminin did not induce significant tyrosine phosphorylation of SHIP2 (Fig. 2); nor did plating on poly-L-lysine-coated surfaces on which cells adhere in a non-integrin-specific manner. These experiments indicated that tyrosine phosphorylation of SHIP2 modification was stimulated through activation of a subset of integrins that interact with collagen I.

**Src inhibitors block SHIP2 tyrosine phosphorylation**

Activation of the cytoplasmic tyrosine kinases, Src and FAK, is an early event during cell attachment to ECM proteins. Several focal adhesion-associated proteins including FAK are substrates of Src and inhibition of Src activation prevents cell adhesion, spreading and migration in several cell types (Cary et al., 1999; Jones et al., 2000; Parise et al., 2000; Schwartz, 2001). Therefore, we tested a possible role for Src family kinases in collagen-I-induced SHIP2 tyrosine phosphorylation. To this end, we made use of specific inhibitors of Src family kinases developed in a separate study (Kraker et al., 2000). When plated on a collagen-I-coated surface in the presence of three different Src-specific inhibitor compounds, PD173956 (#56), PD173958 (#58) and PD180970 (#70) at 1 μM concentration, tyrosine phosphorylation of SHIP2 was...
completely blocked (Fig. 2; Fig. 3A). The inhibitory effect of compound PD173956 was seen in a dose-dependent manner starting at 50 nM (Fig. 3B). Similar results were also obtained with PD180970 (data not shown). Collagen-I-specific SHIP2 tyrosine phosphorylation was also observed in SH-SY5Y, a neuroblastoma cell line, and in Madin Darby canine kidney (MDCK) cells. In SY5Y cells, background levels of SHIP2 tyrosine phosphorylation were lower when cells were cultured on plastic but the collagen-I-induced effect was robust (Fig. 4A). In MDCK cells, cells adherent on plastic displayed strong tyrosine phosphorylation of SHIP2, which was decreased upon detachment and restored during attachment on collagen I (Fig. 4B). It was noted that the extent of SHIP2 modification may vary in a cell-type-dependent manner. In both SH-SY5Y and MDCK cell types, collagen-I-dependent tyrosine phosphorylation was completely blocked in the presence of 1 μM Src inhibitor PD173956 (Fig. 4A,B).

Src kinases do not mediate EGF- or insulin-mediated tyrosine phosphorylation of SHIP2

In HeLa cells, the Src inhibitors did not alter EGF-induced SHIP2 tyrosine phosphorylation (Fig. 5A). IGF-1 modestly induced SHIP2 tyrosine phosphorylation and the effect of Src inhibitors appeared to be partial. In 3T3-L1 adipocytes, pretreatment with Src inhibitor compounds PD173956 and PD180970 failed to prevent SHIP2 tyrosine phosphorylation from occurring in response to insulin (Fig. 5B).

Tyrosines 986-987 are important Src phosphorylation sites

To identify specific tyrosine residues phosphorylated in response to the activation of Src, we examined tyrosine phosphorylation of SHIP2 mutants in which tyrosines conforming to potential phosphorylation site were replaced by phenylalanines. Expression constructs for FLAG-tagged wild-type or SHIP2 mutants were transiently transfected into HeLa cells. Anti-FLAG immunoprecipitates from these cells demonstrated that the YY (986-987) double mutant (YY-FF) in the NPAYY motif was weakly phosphorylated upon re-plating.
Src regulates SHIP2 phosphorylation on collagen I. The same effect was seen upon EGF treatment, indicating that the tyrosines 986-987 can also be phosphorylated by a tyrosine kinase unrelated to Src (Fig. 6A). SHIP2 proteins with mutations in three other tyrosines that were potential phosphorylation sites showed little change in tyrosine phosphorylation when compared with wild-type SHIP2 (Fig. 6B). In vitro phosphorylation assays using purified Src kinase indicated that Src could directly phosphorylate SHIP2. These experiments also showed that the 986-987 YY-FF mutation effectively reduced tyrosine phosphorylation of SHIP2 by purified Src kinase (Fig. 6C). While YY 986-987 sites appear to be the major sites for Src phosphorylation, it is also possible that phosphorylation at these sites may be required for the subsequent phosphorylation of SHIP2 at additional sites.

Exogenous Src kinase stimulates tyrosine phosphorylation of SHIP2 in vivo

In transient transfection experiments shown in Fig. 7A, a constitutively active form of Src induced strong tyrosine phosphorylation of SHIP2. Conversely, a dominant-negative form of Src kinase reduced collagen I-induced SHIP2 tyrosine phosphorylation by about 33-50% (Fig. 7C). Three separate experiments consistently produced this effect in experiments where the transfection efficiency was approximately 30-40%.

Cell spreading on collagen I induces SHIP2 association with Shc via the NPXY motif

The NPXY motif serves as an interaction site for the phosphotyrosine-binding (PTB) domain present in signaling proteins.
Discussion

In this study, we characterize the adhesion-dependent tyrosine phosphorylation of SHIP2 and its role in adhesion and spreading. We show that SHIP2 undergoes tyrosine phosphorylation during attachment and spreading on collagen I but not on fibronectin, collagen IV, laminin or poly-L-lysine. Our experiments indicate that adhesion-dependent SHIP2 tyrosine phosphorylation occurred only in response to signaling from a subset of integrins that interact with collagen I. This is an interesting observation, since the type of collagen as well as the expression pattern of its receptors regulates the behavior of motile cells. For example, integrin α2β1, the collagen I receptor expressed predominantly in epithelial cells, activates synthesis of matrix metalloproteinase MMP1 and MMP13 (Ravanti et al., 1999; Riikonen et al., 1995). Increased expression of collagen receptors α2β1 is observed in highly metastatic melanomas (Klein et al., 1991). Tyrosine...
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phosphorylation of SHIP2 in response to adhesion to collagen I suggests a potential role for SHIP2 in regulation of cell adhesion and motility.

The results described here indicate for the first time that tyrosine phosphorylation of SHIP2 lies downstream of Src kinases during cell adhesion. In vitro phosphorylation experiments further suggest that SHIP2 could be a direct substrate of Src (Fig. 6C). However, we recognize the fact that purified recombinant kinases could potentially phosphorylate non-physiological substrates under such conditions. It is plausible that SHIP2 may be phosphorylated in vivo by another unrelated tyrosine kinase downstream of activated Src such as c-Abl, FAK or Pyk2 (Cary et al., 1999; Plattner et al., 1999). Integrin activation may cause recruitment of SHIP2 to focal contacts and lamellipodia through SH2-mediated interaction with phosphorylated p130Cas (Prasad et al., 2001), where SHIP2 could be phosphorylated.

Although it is still unclear how tyrosine phosphorylation might regulate SHIP2 activity, proper localization of SHIP2, mediated in part by SH2-mediated interactions, appears to be a pre-requisite for this modification [(Taylor et al., 2000) (N.P. and S.J.D., unpublished)]. Tyrosine phosphorylation of SHIP2, may initiate or stabilize its interaction with other yet unidentified signaling molecules. Data presented here support this notion as Src regulates the interaction between SHIP2 and Shc adapter protein mediated through the NPAYY motif of SHIP2. Such interactions may be critical in confining the function of SHIP2 to specific sub-cellular sites. SHIP2 localization to areas where Src and Src substrates reside further underscores its relevance (Dyson et al., 2001; Prasad et al., 2001). Interestingly, SHIP2 is constitutively tyrosine phosphorylated and associated with Shc in Rous-sarcoma-virus-transformed fibroblasts and in BCR-ABL-positive chronic myelogenous leukemia (CML) cells (Habib et al., 1998; Wisniewski et al., 1999). The YY-FF (986-987) motif of SHIP2 is essential for its interaction with Shc and may regulate its function in specific sub-cellular sites.

Fig. 8. SHIP2 associates with Shc via the NPXY motif. (A) Anti-SHIP2 or control (Pre) IPs from HeLa cells that were detached (D) or re-plated for 60 minutes on collagen I (6 μg/cm²; C.I) were blotted with anti-Shc (monoclonal) or anti-SHIP2 antibodies. (B) Anti-SHIP2 or control (Pre) IPs, from HeLa cells that were re-plated for 60 minutes on collagen I (6 μg/cm²; C.I), fibronectin (5 μg/cm²; F), or poly-L-lysine (0.01% solution, 0.5 ml/25 cm²; P-Ly) were blotted with anti-Shc (monoclonal) or anti-SHIP2 antibodies as indicated. Cells were plated in the presence of vehicle control DMSO (–) or 1 μM Src inhibitor compound PD180970 (+). Whole cell lysate was used as a control (WCL). (C) HeLa cells were transiently transfected with wild-type or YY-FF (986-987) mutant SHIP2 expression constructs. 48 hours post-transfection, serum-starved cells were re-plated on collagen I (6 μg/cm²) for 60 minutes. Anti-FLAG (rabbit polyclonal) IPs from these samples were blotted with anti-Shc (monoclonal) or monoclonal anti-FLAG (M2) antibodies. Whole cell lysate was used as a control (WCL). (D) Anti-Shc (monoclonal) or control mouse IgG (MIg) IPs from HeLa cells re-plated for 60 minutes on collagen I (6 μg/cm²; C.I), fibronectin (5 μg/cm²; F), or poly-L-lysine (0.01% solution, 0.5 ml/25 cm²; P-Ly) were blotted with anti-SHIP2 or anti-Shc (polyclonal) antibodies as indicated. Cells were plated in the presence of either vehicle control DMSO (–) or 1 μM Src inhibitor compound PD180970 (+). Whole cell lysate was used as a control (WCL). Small arrows point to the three forms of Shc proteins. The large arrowhead points to SHIP2 protein.
SHIP2 mutant displayed deregulation of lamellipodia formation indicating an important role for interactions mediated through this motif in cell spreading. A role for Shc in cell motility and regulation of actin remodeling is well documented (Collins et al., 1999; Gu et al., 1999; Mauro et al., 1999). Therefore, it is possible that disruption of SHIP2-Shc interaction may have significant impact on actin reorganization as shown by irregular membrane protrusions and actin spikes in cells expressing the YY-FF mutant.

Src is a critical component in adhesion signaling. Src kinase localizes to focal adhesions in fibroblasts and to cell-cell junctions in epithelial cells (Fincham et al., 1996; Owens et al., 2000). Src plays an important role in turnover of focal adhesions and regulates cell motility (Fincham and Frame, 1998). In epithelial cells, activated Src induces disassembly of cell-cell adhesions and promotes ECM-dependent invasion (Owens et al., 2000). As SHIP2 appears to play an important role in cell adhesion and spreading (Dyson et al., 2001; Prasad et al., 2001), phosphorylation of SHIP2 regulated by Src kinases represents a molecular mechanism linking activation of tyrosine kinases associated with integrin signaling to phospholipid metabolism. In support of such a notion, some reports suggest that SHIP1 activity could be regulated through tyrosine phosphorylation by Src family kinases following its relocation to cytoskeleton (Gardai et al., 2002; Giuriato et al., 2000; Lamkin et al., 1997).

Recently, SHIP2 was shown to interact with an actin-binding protein filamin and to regulate membranous actin (Dyson et al., 2001). Filamin serves as a scaffold for RalA, RhoA, Rac and Cdc42 proteins (Ohta et al., 1999). Rho family small GTPases are central regulators of cell adhesion, spreading and migration and are activated by guanine nucleotide exchange factors, GEFs (Ridley, 2001). Many GEFs contain phosphoinositide-binding pleckstrin-homology (PH) domains and are consequently regulated by phosphotyrosyl-phosphatase metabolites. SHIP2 upon localization to focal contacts or to lamellipodia, in conjunction with PI 3-kinase, could cause dynamic waves of lipid second messengers, in turn regulating the activation of guanine nucleotide exchange factors for Rho family proteins in a reversible and dynamic fashion. Such dynamic waves of PtdIns metabolites’ synthesis and degradation during cell spreading and migration has been demonstrated (Haugh et al., 2000). By contrast, SHIP2 activity could also regulate PtdIns(4,5)P_2 levels (Taylor et al., 2000). PtdIns(4,5)P_2, by virtue of its interaction with several actin-binding proteins, plays a critical role in regulation of actin remodeling (Czech, 2000; Takenawa and Itoh, 2001). Taken together, these studies describe a novel pathway involving Src kinases and SHIP2 in the regulation of cytoskeleton, cell adhesion and motility.

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