SHIP1, an SH2 Domain Containing Polynsitol-5-phosphatase, Regulates Migration through Two Critical Tyrosine Residues and Forms a Novel Signaling Complex with DOK1 and CRKL*

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SHIP1 is an SH2 domain containing inositol 5-phosphatase that appears to be a negative regulator of hematopoiesis. The tyrosine kinase oncogene BCR/ABL drastically reduces expression of SHIP1. The major effect of re-expressing SHIP1 in BCR/ABL-transformed cells is reduction of hypermotility. To investigate the potential signaling pathways involving SHIP1 in hematopoietic cells, we overexpressed SHIP1 in a murine BCR/ABL-transformed Ba/F3 cell line and identified SHIP1-associated proteins. SHIP1 was found to form a novel signaling complex with BCR/ABL that includes DOK1 (p62DOK), phosphatidylinositol 3-kinase (PI3K), and CRKL, each of which has been previously shown to regulate migration in diverse cell types. We found that DOK1 binds directly through its PTB domain to SHIP1. Direct interaction of SHIP1 with CRKL was mediated through the CRKL-SH2 domain. Co-precipitation experiments suggest that Tyr917 and Tyr1020 in SHIP1 are likely to mediate interactions with DOK1. In contrast to wild type SHIP1, expression of tyrosine mutant SHIP1 by transient transfection did not alter migration. PI3K was likely linked to this complex by CRKL. Thus, this complex may serve to generate a very specific set of phosphoinositol products, possibly involved in regulating migration. Overall, these data suggest that proteins that interact with SHIP1 through Tyr917 and Tyr1020, such as DOK1 and SHC, are likely to be involved in the regulation of SHIP1 dependent migration.

SHIP1 is a 145-kDa SH2-containing inositol phosphatase which selectively hydrolyzes the 5'-phosphate from inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P4] and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P3]. SHIP2 is a more widely expressed PtdIns(3,4,5)P3-specific 5'-phosphatase related to SHIP1 (3, 4). In addition, a smaller spliced form of SHIP1 has been identified (5). SHIP1 and SHIP2 are transiently tyrosine phosphorylated by growth factor stimulation and by activation of immunoregulatory receptors (1, 6–9).

SHIP1 functions in part by modifying a signaling pathway that is initiated by activation of phosphatidylinositol 3-kinase (PI3K) (10, 11), a lipid kinase with pleiotropic effects (12). SHIP1 was shown to metabolize the PI3K lipid product PtdIns(3,4,5)P3 to phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2). However, it is not entirely clear at this time how such changes in phosphatidylinositol metabolism mediate biological effects. Mice with a disruption of the SHIP1 gene fail to thrive and develop a myeloproliferative disorder with extensive infiltration of myeloid cells in the lung (13). Also, marrow progenitor cells of these mice are hyper-responsive to hematopoietic growth factors (13) and chemokines (14). In cell line models, SHIP1 negatively regulates growth, differentiation, or migration, and it may have an important role in apoptosis (2, 15–17). The enzyme activity of SHIP1 has not been shown to change after receptor activation, suggesting that relocation of SHIP1 to the cell membrane may be critical for signaling (18).

In addition to its enzymatic activity as a regulator of bioactive phospholipids, SHIP1 can also function as an adaptor protein. SHIP1 was originally identified as a SHC-binding protein, an interaction later shown to be mediated by the SH2 domain of SHIP1 (15), and by the protein tyrosine-binding (PTB) domain of SHC (2, 19). GRB2 competes with SHIP1 for SH2 binding to SHC (15, 20) or binds to a C-terminal proline-rich region in SHIP1 through its SH3 domains (1). Another prominent SHIP1-binding protein is the SH2 containing tyrosine phosphatase SHP-2 (21, 22). Deleting the SH2 domain of SHIP1 impairs apoptotic activity and prevents tyrosine phosphorylation (15). It is therefore likely that SHIP1 may be involved in the regulation of several distinct signaling pathways.

We have previously demonstrated that expression of SHIP1 is drastically reduced in cells transformed by the BCR/ABL oncogene (17). BCR/ABL is generated by the t(9,22) (q34;q11) Philadelphia chromosome (Ph) translocation and is the transforming protein in chronic myelogenous leukemia (23). One feature of primary chronic myelogenous leukemia cells is altered adhesion to fibronectin and hypermotility (24, 25). We have shown that re-expression of SHIP1 in BCR/ABL-transformed cells reduces spontaneous Transwell migration (17). The exact mechanism whereby SHIP1 regulates migration in normal and transformed cells is unknown.

In this study, we have used a BCR/ABL-transformed Ba/F3 cell line with inducible SHIP1 expression as a model system to...
investigate the signaling activities of SHIP1. We demonstrate that Tyr<sup>117</sup> and Tyr<sup>1026</sup> in SHIP1 are important for the effects of SHIP1 on migration, and serve as binding sites for DOK1, a signaling protein previously linked to the regulation of migration (26). In addition, we show that the SHIP1-DOK1 complex contains P13K and the unique adapter protein CRKL, also previously linked to migration in hematopoietic cells (27). We propose that SHIP1 regulates migration through the DOK1-CRKL-P13K complex, and that the loss of SHIP1 expression in BCR/ABL-transformed cells results in further activation of migration. Further studies will define the exact role of downstream targets of DOK1 and CRKL as well as their role in regulating migration.

MATERIALS AND METHODS

Cell Culture—The murine hematopoietic line Ba/F3 was grown in RPMI 1640 with 10% (v/v) fetal calf serum and 100 units/ml penicillin (26). For transient expression vectors for GST fusion proteins of the murine SHIP 1 and CRKL SH3 domain, the CRKL SH2, and the CRKL SH3 domain were used. The GST fusion proteins were expressed in Escherichia coli by isopropyl-1-thio-β-D-galactopyranoside induction and isolated from sonicated bacterial lysates using glutathione-Sepharose beads (Amersham Pharma Biotech, Piscataway, NJ) according to the manufacturer’s directions.

Expression Constructs and Transient Expression—Site-directed mutagenesis was performed on the pBluescript-SHIP1 plasmid (2) using the QuikChange Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer’s directions. Complementary overlapping oligonucleotides were synthesized for altering Tyr<sup>117</sup> to Phe and Tyr<sup>1026</sup> to Phe. Relevant regions were sequenced to confirm successful mutagenesis. The murine SHIP1 wild type and mutant cDNA was subcloned into the pEGFP-C1 expression plasmid (Clontech Laboratories, Palo Alto, CA). The pTRE-SHIP1 expression construct was used for transfection into Ba/F3.p210.TetON cells. For Transwell migration experiments pTRE-SHIP1 constructs were co-transfected with an enhanced green fluorescence protein (EGFP) expression construct (EGFP-C1, ClONTECH Laboratories). EGFP positive cells were sorted 1 day after transfection using a Coulter Epics Altra or Elite flow cytometer (Coulter Corp. Miami, FL). SHIP1 expression was induced 1 day after transfection by treatment with 1 μg/ml doxycycline.

Transwell Migration Assay—The lower chamber of a Transwell plate (8-μm pore size polycarbonate membrane, Corning Costar Corp., Cambridge, MA) was filled with 600 μl of starvation media (0% (v/v) bovine serum albumin in RPMI 1640). Cells were counted using a Coulter particle counter (Coulter Counter Z2, Beckman Coulter, Fullerton, CA) and resuspended at 2 × 10<sup>5</sup> cells/ml in starvation media. 100 μl of this cell suspension was transferred to the upper chamber. The medium contained either doxycycline (1 μg/ml) or no stimulus in the control samples. After 2.5 h, cells in the lower compartment were resuspended and counted using a Coulter particle counter. The spontaneous Transwell migration of cells was expressed as a “migration index” (number of migrating cells treated with doxycycline divided by the number of migrating cells left untreated). The standard error of the mean was calculated from the migration index of independently performed experiments. The statistical significance of the data was analyzed using the Student’s t test.

RESULTS

SHIP1 Forms Complexes with Multiple Proteins in BCR/ABL Transformed Ba/F3 Cells Re-expressing SHIP1—We and others have previously shown that SHIP1 is a negative regulator of cell migration (14, 17). However, none of the known signaling pathways associated with SHIP1 have been directly linked to this process. Furthermore, we have shown that loss of SHIP1 expression in BCR/ABL-transformed cells enhances migration (17). To investigate the molecular mechanisms that regulate migration in these cells, we looked for novel SHIP1-associated signaling complexes in BCR/ABL-transformed cells. To visualize SHIP1-containing complexes, it was helpful to overexpress SHIP1 in BCR/ABL-transformed cells. A doxycycline inducible expression system was used to increase SHIP1 expression by severalfold in Ba/F3.p210.TetON cells compared with flow-through cells from Ba/F3.p210.TRE cells (Fig. 1A, left panel). However, there was slightly increased migration of SHIP1 in untreated Ba/F3.p210.TRE-SHIP cells compared with untreated Ba/F3.p210.TRE cells, likely indicating that the promoter is leaky. Expression of the SHIP1 protein in Ba/F3.p210 cells led to the co-immunoprecipitation of additional tyrosine-phosphorylated proteins with an apparent molecular mass of 210, 190, 140, 120, and 50–70 kDa.
Association of tyrosine-phosphorylated proteins with SHIP1 of comparable molecular mass could also be observed in similar experiments with the parental Ba/F3.p210 cells when the blot was exposed for a long time (22).

SHIP1 Binds to p62 DOK1 in BCR/ABL-transformed Cells—The 62-kDa tyrosine-phosphorylated protein that co-immunoprecipitated with SHIP1 was identified as DOK1 (Fig. 1A, left bottom panel). These results were confirmed by immunoprecipitation with anti-DOK1 followed by immunoblotting with anti-SHIP1 using lysates of Ba/F3.p210 cells re-expressing SHIP1 (Fig. 1A, right panel). We also found PI3K co-immunoprecipitating with SHIP1 in doxycycline-treated Ba/F3.p210.pTRE-
SHIP1 (Fig. 1A, left bottom panel). In contrast, there was only a very small amount of PI3K found to be constitutively associated with DOK1 when the blot was exposed for a long time (data not shown), indicating that inducible interaction of PI3K with SHIP1 was not mediated through interaction with DOK1. Immunoprecipitations of SHIP1 and DOK1 showed overlapping phosphotyrosine patterns, suggesting that both proteins were in the same signaling complex in these cells.

The above results suggest the potential formation of a multimeric signaling complex including SHIP1 and DOK1. Since SHIP1 has one SH2 domain and DOK1 has a PTB domain, we sought to determine the mechanism of binding of SHIP1 to DOK1 using GST fusion proteins containing the phosphotyrosine interaction domains of each protein. Similar to the previous experiments, we used lysates of untreated and doxycycline-treated Ba/F3.p210.pTRE-SHIP and Ba/F3.p210.pTRE cells to test the interaction of SHIP1 with DOK1 (Fig. 1B). The SH2 domain of SHIP1 precipitated both SHIP1 and DOK1 from lysates of doxycycline-treated Ba/F3.p210.pTRE-SHIP and a small amount of DOK1 from untreated cells. The BCR/ABL oncoprotein itself was found to precipitate with the SHIP1 SH2 domain in untreated as well as in doxycycline-treated Ba/F3.p210.pTRE and Ba/F3.p210.pTRE-SHIP cells. In contrast, the PTB domain of DOK1 precipitated SHIP1 and a small amount of BCR/ABL only from lysates of doxycycline-treated Ba/F3.p210.pTRE-SHIP cells. Neither BCR/ABL, SHIP1, nor DOK1 were found to bind to GST alone.

The in vitro GST fusion protein precipitations with SHIP1 and DOK1 did not indicate whether binding of the SH2 or PTB domains was direct or indirect. A protein overlay assay was used to identify direct in vitro interactions. Cellular lysates from untreated and doxycycline-treated Ba/F3.p210.pTRE-SHIP cells were used for immunoprecipitations with anti-SHIP1 and anti-DOK1 antibodies. GST protein alone did not bind to SHIP1 or DOK1 proteins in immunoprecipitations (Fig. 1C, top left panel). Direct binding of a single 145-kDa protein band in SHIP1 immunoprecipitates using the GST-DOK1-PTB protein as a probe was found in Ba/F3.p210 cells re-expressing SHIP1 (Fig. 1C, top right panel). A weak interaction between the SHIP1 SH2 domain and DOK1 was detected in SHIP1 overexpressing cells, but the SHIP1-SH2 domain did not bind to SHIP1 itself (Fig. 1C, bottom right panel). We also observed direct in vitro binding of the SHIP1 SH2 domain to BCR/ABL that was increased in SHIP1 over-expressing cells. These data suggest that overexpression of SHIP1 protects the dephosphorylation of DOK1 and BCR/ABL on a site that is important for binding the SHIP1-SH2 domain. Thus, DOK1 is linked through its PTB domain to SHIP1, whereas the SH2 domain of SHIP1 is only involved in a weak interaction with DOK1.

Since SHIP1 has two binding sites for the PTB domain of SHC, we also tested if these sites regulate binding to the DOK1-PTB domain. Full-length SHIP1 and the SHC-binding mutants of SHIP1 containing the Y917F and Y1020F substitutions in the SHC-binding site (28) were expressed in Ba/F3.p210.TetON cells. The cells were treated with doxycycline to induce SHIP1 expression. Cells transfected with the SHIP1 containing vectors expressed high levels of SHIP1 compared with cells transfected with the empty vector (Fig. 1D, left panel). Using the DOK1-PTB domain, a significant amount of SHIP1 was found to precipitate from cells re-expressing SHIP1, but not from cells transfected with the empty vector (Fig. 1D, right panel). The amount of SHIP1 tyrosine mutants precipitating with the DOK1-PTB domain was reduced significantly when compared with wild type SHIP1. These data are consistent with previous findings demonstrating that optimal binding of the SHC PTB domain to SHIP1 is reduced but not abolished by mutating either tyrosine (28).

**SHIP1 and CRKL Are Associated in BCR/ABL-transformed Ba/F3 Cells Re-expressing SHIP1—** Since DOK1 has previously been shown to co-precipitate with CRKL in BCR/ABL-transformed cells (29), we also asked if CRKL was found in the complex with SHIP1. Ba/F3.p210.pTRE and Ba/F3.p210.pTRE-SHIP cells were either left untreated or treated with doxycycline and CRKL protein was immunoprecipitated from whole cell lysate. SHIP1 was found to co-precipitate with CRKL only when re-expressed in Ba/F3.p210.pTRE-SHIP (Fig. 2A). We also found PI3K in this complex which we had previously demonstrated to bind constitutively to the CRKL SH3 domain (30). SHIP1 was not found in a complex with CRKL in Ba/F3.p210.pTRE or unstimulated Ba/F3 cells (not shown). Next, the molecular interactions of CRKL with SHIP1 using GST fusion proteins of the SHIP-SH2, CRKL-SH2, or CRKL-SH3 domains were determined. We did not observe significant co-precipitation of CRKL in lysates of Ba/F3.p210 cells (not shown). In contrast, SHIP1 co-precipitated with the CRKL-SH2 domain when re-expressed in Ba/F3.p210.pTRE-SHIP cells (Fig. 2B). A control, we also showed binding of the CRKL-SH3 domain to the p85 regulatory subunit of PI3K (30) and binding of the CRKL-SH2 domain to p110* (31). Re-expression of SHIP1 did not significantly alter either interaction. Since SHIP1 contains Tyr-XX-Pro motifs, potential binding sites for the CRKL SH2 domain, we also determined if there was direct in vitro interaction between SHIP1 and CRKL. GST or a GST-CRKL fusion proteins were used for protein overlay experiments with CRKL and CBL immunoprecipitations of untreated and doxycycline-treated Ba/F3.p210.pTRE-SHIP cells. The CRKL-SH2 domain was found to bind to SHIP1 immunoprecipitations to a 145-kDa protein in cells re-expressing SHIP1 (Fig. 2C). In addition, the CRKL SH2 domain was found to bind to proteins with an apparent molecular mass of 55 and 70 kDa in SHIP1 immunoprecipitations. As a positive control, the CRKL SH2 domain bound to CBL in CBL immunoprecipitations. These data suggest that there was direct in vitro binding of CRKL through its SH2 domain to SHIP1.

**SHIP1 Binds to DOK1 and CRKL in Ba/F3 Cells Transformed by TEL/ABL and v-Abl—** We next investigated the potential interaction of SHIP1 with DOK1 and CRKL in cells that are transformed by activated forms of Abl, different from BCR/ABL, including TEL/ABL and v-Abl. Cells transformed by activated forms of Abl have increased levels of tyrosine-phosphorylated proteins compared with untransformed cells. Both Ba/F3.TEL/ABL- and Ba/F3.v-Abl-transformed cells had higher levels of SHIP1 compared with Ba/F3.p210 cells (Fig. 3A). Transformation of Ba/F3 cells by TEL/ABL and v-Abl induces growth factor independence and requires ABL kinase activity. We have shown before that treatment of Ba/F3 cells transformed by activated ABL kinases with the ABL kinase inhibitor STI571 results in re-expression of SHIP1 and returns these cells to growth factor dependence. Consistent with these findings, we found that STI571 treatment reduced the Transwell migration of TEL/ABL cells by 51.1% (n = 3) and v-Abl cells by 47.9% (n = 3).

Tyrosine phosphorylation of the 145-kDa protein SHIP1 was increased in cells transformed by TEL/ABL and v-Abl compared with Ba/F3 cells (Fig. 3B). In some experiments, we also detected low level tyrosine phosphorylation of SHIP1 in growth factor-deprived Ba/F3 cells. In addition, SHIP1 co-precipitated with major tyrosine-phosphorylated proteins with an apparent molecular mass of 170, 120, and 50–70 kDa in TEL/ABL-transformed cells and 150, 120, and 50–70 kDa in v-Abl-transformed cells. ABL immunoblotting also demonstrated that c-
ABL and the oncoproteins TEL/ABL and v-Abl were found in this complex.

Next, we also tested if DOK1 and CRKL were in a complex with SHIP1 in TEL/ABL and v-Abl-transformed cells. Consistent with the previous results, we found co-precipitation of the 145-kDa phosphotyrosine protein SHIP1 with DOK1 (Fig. 3C, left panel). The amount of SHIP1 associated with both proteins was increased in the transformed cell lines compared with the untransformed Ba/F3 cells and it correlated to the amount of SHIP1 expressed in either Ba/F3, Ba/F3.p210, Ba/F3.TEL-ABL and Ba/F3.v-Abl cells. However, we found only a very small amount of SHIP1 associated with CRKL in v-Abl but not TEL-ABL-transformed cells (Fig. 3C, right panel).

Tyrosines 917 and 1020 in SHIP1 Regulate Spontaneous Transwell Migration—BCR/ABL-transformed Ba/F3 cells demonstrate a significant level of spontaneous migration which can be reduced by re-expression of SHIP1. Using the above described doxycycline inducible expression system, we co-transfected SHIP1 and EGFP expression vectors and sorted for EGFP positive cells 24 h after transfection. SHIP1 and SHIP1 mutant levels were increased severalfold after doxycycline treatment in transiently transfected cells (Fig. 4A). The effect on Transwell migration of EGFP sorted wild type SHIP, SHIP-Y917F, and SHIP-Y1020F mutant transfected cells was investigated after doxycycline treatment and compared with untreated cells. We had previously shown that doxycycline...
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Treatment alone does not alter Transwell migration (17). The migration index of 0.74 ($n = 4, p < 0.01$) in cells transiently expressing wild type SHIP1 demonstrates a significant decrease in spontaneous migration (Fig. 4B). However, the decrease was smaller than the previously described decrease in stably transfected cell lines after SHIP1 expression (17). In contrast, SHIP-Y917F and SHIP-Y1020F expression in Ba/F3.p210.TetON cells induced a small but not significant decrease in Transwell migration. The mean of the migration index of 0.74 ($n = 4, p = 0.1$) and 0.94 for SHIP-Y1020F ($n = 3, p = 0.2$) expressing cells. These data suggest that Tyr917 and Tyr1020 in SHIP1 are likely to be involved in regulating SHIP1-dependent migration.

Discussion

Expression of the phosphatidylinositol-5-phosphatase SHIP1 is rapidly and reversibly down-regulated by BCR/ABL and this requires ABL kinase activity (17). Down-regulation of SHIP1 by BCR/ABL is of particular significance because disruption of the SHIP1 gene by gene targeting results in a myeloproliferative disorder in mice (13). We have shown that re-expression of SHIP1 in BCR/ABL-transformed Ba/F3 cells reduces hypermotility (17), a characteristic of BCR/ABL transformation (25). However, the mechanism whereby SHIP1 expression regulates migration is unknown.

In this report, we demonstrate the presence of protein complexes that contain SHIP1, DOK1, and CRKL in a murine BCR/ABL-transformed hematopoietic cell line. CRKL is constitutively associated with PI3K which is also recruited to this complex. Expression of SHIP1 is reduced in cell lines transformed by BCR/ABL, and the remaining SHIP1 is heavily tyrosine phosphorylated (22). In untransformed cells SHIP1, CRKL, and DOK1 are not spontaneously tyrosine phosphorylated and do not co-immunoprecipitate, demonstrating that this complex is altered by BCR/ABL. DOK1 and CRKL are also constitutively tyrosine phosphorylated in BCR/ABL-transformed cells and the interaction with SHIP1 is increased considerably following re-expression of SHIP1.

DOK1 (for downstream of kinase) was originally identified as a tyrosine-phosphorylated protein in cells transformed by oncogenic tyrosine kinases. The DOK1 cDNA was cloned from cells transformed by activated forms of ABL and identified as the 62-kDa Ras GTPase-activating protein (RasGAP)-associated protein (32, 33). DOK1 belongs to a family of related proteins that also includes DOK2 (34) and DOK3 (35). DOK1 has been found to be tyrosine phosphorylated in response to steel factor (32, 36), epidermal growth factor (37), or insulin (26, 38) and other stimuli. DOK1 contains a pleckstrin homology domain that facilitates interaction with phosphoinositides and a PTB domain that is likely to interact with Asn-Pro-Xxx-phospho-Tyr motifs (39, 40). The major phosphotyrosine site in DOK1 is thought to be a negative regulator of RAS activation (43). Tyrosine phosphorylation of DOK1 is also likely to be required for its inhibitory effect on RasGAP activity (43). Overexpression of DOK1 enhances insulin-induced cell migration and requires that the pleckstrin homology domain and the major phosphotyrosine site (Tyr361, murine sequence) of DOK1 be intact (26). This is of interest, since we show interaction of SHIP1 and DOK1 in BCR/ABL-transformed cells, suggesting that both molecules act in the same signaling pathway. We have demonstrated direct in vitro binding of DOK1 to SHIP1 through the DOK1 PTB domain. Interestingly, DOK3 was also recently found in a signaling complex with SHIP1 (35). During preparation of this article others also reported that DOK1 is found in a signaling complex with SHIP1 (41, 42). The exact function of DOK1 is unknown but it has been demonstrated that tyrosine phosphorylation of DOK1 regulates its binding to RasGAP (32, 33, 43). Tyrosine phosphorylation of DOK1 is also likely to be required for its inhibitory effect on RasGAP activity (43). Overexpression of DOK1 enhances insulin-induced cell migration and requires that the pleckstrin homology domain and the major phosphotyrosine site of DOK1 be intact (26).

This is of interest, since we show interaction of SHIP1 and DOK1 in BCR/ABL-transformed cells, suggesting that both molecules act in the same signaling pathway. We have demonstrated that SHIP1 regulates migration (17), and here demonstrate that Tyr917 and Tyr1020 major regulatory sites for this effect, also regulate binding to DOK1. Overexpression of SHIP1 in the Ph+ cell line K562 has been shown to decrease synthesis of hemoglobin protein and ε-globin mRNA in response to hemin, an inducer of erythroid differentiation (44). This process was also inhibited by mutating Tyr1020 to phenylalanine. Interestingly, this phosphotyrosine in SHIP1 is also a binding site for the adapter protein SHC and it is therefore likely that SHC and DOK1 compete for binding to SHIP1 (28). Whereas DOK1 is thought to be a negative regulator of Ras activation (43). SHC is believed to be involved in activation of Ras by interacting with the GRB2-SOS complex (45). It is uncertain if activation of Ras is sufficient to regulate Transwell migration in these cells. Nevertheless, it has been suggested that Ras can affect migration and motility in different in vitro models (46–49).
The other adapter protein found in a complex with SHIP1 was CRKL, an SH2/SH3 domain containing phosphotyrosine protein. CRKL was originally described as a major tyrosine-phosphorylated protein in stable phase chronic myelogenous leukemia neutrophils. Cloning of the CRKL cDNA revealed that it belonged to the CRK family of adapter proteins that also includes v-CRK, CRK-I, and CRK-II (50–52). CRKL and CRK have been described to be involved in oncogenic and normal signaling and both can interact constitutively or transiently with various signaling proteins (53). For example, CRKL can bind to tyrosine-phosphorylated HEF1 after integrin ligation through its SH2 domain (54) or CRKL forms a constitutive complex with c-ABL through its SH3 domain (31). Here we demonstrate that the CRKL SH2 domain can bind directly to SHIP1, likely through a phospho-Tyr-Xxx-Xxx-Pro site within the ETS family transcription factor gene TEL with c-ABL and v-Crk to induce migration of Ba/F3 cells and this also required the CRKL SH2 domain (27).

The role of the SHIP-CRKL-P3K complex in BCR/ABL transformation is of interest, since there is striking evidence that P3K is important for transformation by BCR/ABL (56). It will therefore also be important to evaluate the role of CRKL in the regulation of P3K or SHIP1. An important question in signaling involved in P3K is how specificity is obtained, since P3K is involved in the generation of several different bioactive 3-phosphorylated phospholipids that regulated different functions and interact with different proteins (12). It is possible that CRKL brings P3K to the proximity of SHIP1 at the cell membrane to generate certain levels of PtdIns(3,4,5)P3 and PtdIns(3,4)P2 and therefore generate a transient and defined signaling focal point of bioactive phospholipids. Such a colocalization of P3K with a specific phosphatase to a subcellular compartment could well mediate specificity in P3K signaling and lead to the activation of defined signaling pathways. Nevertheless, it is also possible that CRKL recruits other signaling molecules to SHIP1 and regulates a function in addition to the contribution of this signaling complex to transformation.

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