Src Homology 2-containing Inositol 5-Phosphatase 1 Binds to the Multifunctional Docking Site of c-Met and Potentiates Hepatocyte Growth Factor-induced Branching Tubulogenesis*

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Hepatocyte growth factor (HGF)/scatter factor is a multifunctional cytokine that induces mitogenesis, motility, and morphogenesis in epithelial, endothelial, and neuronal cells. The receptor for HGF/scatter factor was identified as c-Met tyrosine kinase, and activation of the receptor induces multiple signaling cascades. To gain further insight into c-Met-mediated multiple events at a molecular level, we isolated several signaling molecules including a novel binding partner of c-Met, SH2 domain-containing inositol 5-phosphatase 1 (SHIP-1). Western blot analysis revealed that SHIP-1 is expressed in the epithelial cell line, Madin-Darby canine kidney (MDCK) cells. SHIP-1 binds at phosphotyrosine 1356 at the multifunctional docking site. Because a number of signaling molecules such as Grb2, phosphatidylinositol 3-kinase, and Gab1 bind to the multifunctional docking site, we further performed an in vitro competition study using glutathione S-transferase- or His-tagged signaling molecules with c-Met tyrosine kinase. Our binding study revealed that SHIP-1, Grb2, and Gab1 bound preferentially over phosphatidylinositol 3-kinase. Surprisingly, MDCK cells that overexpress SHIP-1 demonstrated branching tubulogenesis within 2 days after HGF treatment, whereas wild-type MDCK cells showed tubulogenesis only after 6 days following treatment without altering cell scattering or cell growth potency. Furthermore, overexpression of a mutant SHIP-1 lacking catalytic activity impaired HGF-mediated branching tubulogenesis.

Met tyrosine kinase is the receptor for hepatocyte growth factor (HGF)†/scatter factor (1, 2), and signaling via the binding of this receptor to a ligand has been shown to affect a wide range of biological activities, including angiogenesis (3, 4), cellular motility (5), growth (6, 7), invasion (8–10), and morphological differentiation including neuronal branching (11–14). c-Met also plays a role in normal embryological development (15–17), tissue regeneration (18), and wound healing (19). Furthermore, HGF also cooperates with nerve growth factor to enhance axonal outgrowth from cultured dorsal root ganglion neurons (14).

Binding of the growth factors causes activation of their inherent receptor tyrosine kinases leading to autophosphorylation of the cytoplasmic domains at multiple tyrosine residues. The newly formed phosphotyrosines constitute binding sites for the Src homology 2 (SH2) domain- and/or phosphotyrosine binding domain-containing cytoplasmic proteins. Met tyrosine kinase was reported to interact with several substrates including the growth factor receptor-bound protein (Grb) 2 (20), STAT3 (21), the p85 subunit of phosphatidylinositol (PI) 3-kinase (22), Shc (23), phospholipase C-γ (PLCγ) (20), c-Src (20), and Gab1 (24). Interestingly, all these proteins associate with c-Met via a multiple substrate binding site. The c-Met-mediated signaling was well studied in the epithelial cell system, MDCK cells. On planar culture surfaces, sub-confluent MDCK cells normally form coherent islands of relatively flattened cells. HGF has been shown to alter this morphology by promoting the initial expansion of colonies followed by dispersion of the cells that make up these colonies and an increase in their motility (cell scattering) in liquid cell culture medium (25). The activation of a number of tyrosine kinase receptors including Neu, Ros epidermal growth factor receptor, and keratinocyte growth factor receptor induced cell scattering in MDCK cells (26). Royal and Park (27) reported that PI 3-kinase and Ras are required for cell scattering. In addition, stimulation with HGF induces the formation of tubular structures from MDCK cells grown in a three-dimensional collagen matrix (11). For this, the epithelial cells are grown for several days in collagen, in which they form cysts. When HGF/scatter factor is added, individual cells dissociate and form continuous tubules (28). It has been reported that a number of signaling molecules including Gab1 (24), Grb2 (29), PLCγ (30), and STAT3 (21) are required for this process.

The underlying mechanisms of these multiple molecular events, however, are still poorly understood. In the present study we identified SH2-containing inositol 5-phosphatase (SHIP) 1 as a novel binding partner of c-Met by yeast two-hybrid screening using a rat brain library. SHIP-1 binds to one of the tyrosine residues at the multiple substrate binding site, 1356pYNV, which is also the binding site for Grb2. Interestingly, the YVNV motif is identical to the common binding site of Shc to SHIP and Grb2. Furthermore, we show that PI

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§ The abbreviations used are: HGF, hepatocyte growth factor; SH2, Src homology 2; Grb, growth factor receptor-bound protein; PI, phosphatidylinositol; PLCγ, phospholipase C-γ; MDCK, Madin-Darby canine kidney; SHIP, SH2-containing inositol 5-phosphatase; pYV, phosphotyrosine; GST, glutathione S-transferase; FCS, fetal calf serum; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; MBD, Met binding domain.
3-kinase was not able to bind c-Met in the presence of Grb2, Gab1, or SHIP-1. Overexpression of SHIP-1 in MDCK cells drastically enhanced the branching potency of c-Met without affecting the mitogenic and scattering potency. Furthermore, overexpression of a mutant SHIP-1 lacking catalytic activity impaired HGF-mediated branching tubulogenesis.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions, Transfection, and Yeast Two-hybrid Screening**—The construction of LexA fusion genes encoding the cytoplasmic domains of c-Met, c-PI3, TrkA, insulin receptor, and c-Kit downstream of LexA and the expression in Saccharomyces cerevisiae strain L40 were described previously (24, 31, 32). Met mutants containing tyrosine/phenylalanine replacements have been described (24). A single colony, selected for expression of the LexA fusion protein, was tested for auto-phosphorylation and used for transformation with the VP16 cDNA library derived from an 8-day rat brain (33). GST-Grb2, GST-SHIP, GST-Gab1, GST/PI 3-kinase fusion proteins were generated in the pGex system (Amersham Pharmacia Biotech). His-tagged SHIP, Grb2, and PI 3-kinase were generated in pQE30 (Qiagen, Hilden, Germany). Myc-tagged wild-type and mutant SHIP-1 were generated using pcDNA3.1Mye-His (Invitrogen, Carlsbad, CA).

**Binding Assay Using the Two-hybrid System**—The qualitative and quantitative evaluations of various two-hybrid protein/protein interactions were described previously (31).

**Cells and Antibodies**—MDCK cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS. HGF was from Sigma. Scattering and branching tubulogenesis assays were performed as described by Gual et al. (30). Monoclonal antibodies against phosphotyrosine (4G10) and c-Met were from Upstate Biotechnology Inc. (Lake Placid, NY). Monoclonal antibody against Myc and rabbit IgG against c-Met and SHIP-1 were from Santa Cruz Biotechnology (Santa Cruz, CA).

**Tyrosine Kinase Assays and Immunoblotting**—These assays were performed as published (31, 34).

**Coimmunoprecipitation Study**—After incubation for 8 h with medium containing 0.02% FCS, MDCK cells were stimulated with HGF (60 ng/ml) for 5, 10, or 30 min. Cells were extracted with lysis buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% trisylol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 400 μM sodium orthovanadate. One aliquot of each lysate (5 × 10⁶ cells) was incubated for 16 h at 4 °C with anti-SHIP or anti-Met antibody preabsorbed on Staphylococcus aureus Cowan I strain. After washing, materials were analyzed by SDS-PAGE and by immunoblot analyses using the appropriate antibodies.

**Binding of Cellular Proteins to GST Fusion Proteins and His-tagged Proteins**—GST fusion proteins and His-tagged proteins were produced as recommended by the manufacturer. Purified GST fusion proteins were bound for 1 h at 4 °C to glutathione-agarose beads (40 μl slurry; Amersham Pharmacia Biotech) suspended in the binding buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% trisylol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 20 mg/ml bovine serum albumin, and 200 μM sodium orthovanadate). After incubation with 32P-labeled autophosphorylated c-Met overnight, beads were washed five times with binding buffer, and pellets were analyzed by SDS-PAGE.

**RESULTS**

**SHIP-1 Associates with c-Met Tyrosine Kinase**—To identify neuronal proteins that specifically interact with the cytoplasmic domain of c-Met, we employed a yeast two-hybrid screening using an 8 day-old rat brain library (24, 33, 35). A total of 800 cDNA clones of various length were obtained, together encoding seven different proteins. Five of these proteins were previously identified as c-Met-binding proteins including Gab1, PI 3-kinase, Grb2, PLCγ, and c-Src. Furthermore, we detected the interaction with a novel c-Met-interactive zinc finger-containing protein; however, the biological significance of this association remains to be studied. In addition to these molecules, we isolated SHIP-1 as a novel c-Met binding partner.

SHIP-1 was originally identified as a signaling molecule in cytokine-stimulated hematopoietic cells such as macrophage colony-stimulating factor-stimulated cells (36) and has been clearly demonstrated to participate in the signaling pathways in hematopoietic cell systems (37). To determine whether SHIP-1 is a relevant signaling molecule in other cell systems, we next analyzed SHIP-1 expression in various cell lines such as colon carcinoma CACO-2; MDCK cells; two human breast carcinoma cell lines, EFM-19 and EFM-192A; and Raji cells. For a control, we applied a cell lysate derived from myeloid progenitor cell line FDC-P1Mac11 cells (38). In agreement with a previous report (36), SHIP-1 is expressed at high levels in myeloid progenitor cell line FDC-P1Mac11 cells. In addition, SHIP-1 was also detected in the epithelial cell lines, MDCK, both human breast carcinoma cell lines, and Raji cells (Fig. 1).

To demonstrate the protein/protein interaction between SHIP and c-Met in vivo, MDCK cells were incubated for 8 h with medium containing 0.02% FCS, then stimulated with HGF (60 ng/ml) for 5, 10, and 30 min, and studied by immunoprecipitation. Aliquots of 5 × 10⁶ cells from each preparation were lysed for immunoprecipitations using the SHIP-specific or c-Met-specific antibodies. Precipitated material was analyzed for the presence of c-Met or SHIP by SDS-PAGE and Western blotting using SHIP-, c-Met-, or phosphotyrosine (4G10)-specific antibodies (Fig. 2). Following HGF stimulation, tyrosine phosphorylation of c-Met was observed throughout the experiment. Prior to HGF stimulation (0 min), no c-Met was detectable on the SHIP-specific immune complexes. In contrast, between 5 and 30 min after HGF stimulation, c-Met was coprecipitated with SHIP (Fig. 2).

The Phosphorylated Tyrosine 1356 Provides the Binding Site for SHIP-1—To determine whether the SHIP-1-c-Met interaction relied on the presence of particular phosphotyrosine residues of c-Met, we employed a set of c-Met mutants in which...
established binding sites for defined binding partners were destroyed (24). These mutants included Y1349F (Y14F), Y1356F (Y15F), the double mutant Y14F/Y15F, and the kinase negative mutant K1110A. We examined the binding of these mutants with the SH2 domain of SHIP, PI 3-kinase, Grb2, Grb10, c-Src, or PLCγ, the MBD domain of Gab1, or the phosphorysorine binding domain of Shc, as VP16 fusion proteins in YRN974 (31). Aliquots of 10,000 cells of four independent transformants were analyzed for fluorescence intensity using a Becton Dickinson FACScan flow cytometer. Values represent the means obtained in four independent experiments.

Fig. 3. Phosphotyrosine 1356 of c-Met is a binding site of SHIP. Interaction of SHIP, PI 3-kinase, Grb2, and Gab1 with c-Met mutants carrying tyrosine/phenylalanine or lysine/alanine replacements as Y1349F (Y14F), Y1356F (Y15F), double mutant Y14F/Y15F, and kinase negative mutant K1110A. These mutants were coexpressed with the SH2 domain of SHIP, PI 3-kinase, Grb2, Grb10, c-Src, or PLCγ, the MBD domain of Gab1, or the phosphotyrosine binding domain of Shc, as VP16 fusion proteins in YRN974 (31). Aliquots of 10,000 cells of four independent transformants were analyzed for fluorescence intensity using a Becton Dickinson FACScan flow cytometer.

Table I

| Tyrosine kinase substrate | SHIP binding site | PI 3-kinase binding site | Grb2 binding site |
|---------------------------|-------------------|--------------------------|-------------------|
| c-Met                     | pYVNV             | pYVHV (20)               | pYVYNV (20, 56)   |
| She                       | pYVNV (44)        |                          |                   |
| c-Fms                     | ?                 | pYVEM (39)               | pYKNI (58)        |
| c-Kit                     | pYMDM (40)        | pYKNL (59)               |                   |
| Insulin receptor          | pYHTM (41)        |                          |                   |
c-Src, and Shc bind to both Y14F (Y1349F) and Y15F (Y1356F) (Fig. 3).

SHIP Binds to c-Met and c-Fms but Not to c-Kit, TrkA, or the Insulin Receptor—Signaling molecules that bind to the multifunctional docking site such as PI 3-kinase, Shc, and PLCγ associate with most receptor tyrosine kinases. Because SHIP-1 binds at the same site in c-Met with these molecules, we performed similar binding analyses with four additional activated receptor tyrosine kinases including c-Fms, c-Kit, TrkA, and the insulin receptor. Western blotting with anti-phosphotyrosine antibody clearly demonstrated that these receptor moieties were phosphorylated on tyrosine in the yeast two-hybrid assay (data not shown). As expected, c-Fms binds to SHIP-1; however, SHIP-1 did not bind other tyrosine kinase receptors such as c-Kit, TrkA, or the insulin receptor. Western blotting with anti-phosphotyrosine antibody clearly demonstrated that these receptor moieties were phosphorylated on tyrosine in the yeast two-hybrid assay (data not shown). As expected, c-Fms binds to SHIP-1; however, SHIP-1 did not bind other tyrosine kinase receptors such as c-Kit, TrkA, or the insulin receptor (Fig. 4). In agreement with previous data (39–41), PI 3-kinase binds to c-Fms, c-Kit, TrkA, and the insulin receptor. Western blotting with anti-phosphotyrosine antibody clearly demonstrated that these receptor moieties were phosphorylated on tyrosine in the yeast two-hybrid assay (data not shown). As expected, c-Fms binds to SHIP-1; however, SHIP-1 did not bind other tyrosine kinase receptors such as c-Kit, TrkA, or the insulin receptor (Fig. 4). In agreement with previous data (39–41), PI 3-kinase binds to c-Fms, c-Kit, and the insulin receptor, the sequences of which contain the typical consensus motif, pYXXM, for the PI 3-kinase binding site (42). It is noteworthy that both of the PI 3-kinase binding sites of c-Met, tyrosine 1349 and 1356, and their following amino acid sequences are different from this motif (Table I).

Hierarchical Binding of Grb2, Gab1, SHIP, and PI 3-Kinase to the Multifunctional Docking Site of c-Met—The multifunctional docking site of c-Met provides binding sites for many substrates including PI 3-kinase, Grb2, Gab1, and SHIP. As shown in Fig. 3, PI 3-kinase and Gab1 bind at both phosphotyrosine 1349 and phosphotyrosine 1356, whereas SHIP and Grb2 bind only at phosphotyrosine 1356. This observation raises the question whether two of these molecules bind to c-Met simultaneously or sequentially. To answer this question, we have generated the GST-SH2 domain of Grb2 (Grb2(SH2)), p85 of PI 3-kinase (PI 3-kinase(SH2)), and SHIP (SHIP(SH2)) fusion proteins and GST-MBD of Gab1 (Gab1(MBD)), which were incubated with tyrosine-autophosphorylated c-Met in the presence and absence of His-tagged PI 3-kinase(SH2), Grb2(SH2), or SHIP(SH2). Firstly, we compared the binding of c-Met to PI 3-kinase and SHIP. When equal amounts of both molecules were incubated with c-Met, SHIP bound predominantly to c-Met. Secondly, Grb2 bound to c-Met preferentially over PI 3-kinase and Gab1 (Fig. 5). Thirdly, SHIP did not compete with the c-Met-Gab1 interaction. Taken together, these results indicate that when phosphotyrosine 1356 binds to either Grb2 or SHIP, phosphotyrosine 1349 does not provide a binding site for PI 3-kinase and that when Grb2 binds to phosphotyrosine 1356, even Gab1 does not bind to c-Met, suggesting that in vivo these molecules may bind to a single molecule of c-Met sequentially.

Overexpression of SHIP-1 in MDCK Cells Dramatically Potentiated Branching Tubulogenesis Induced by c-Met without Altering HGF-stimulated Cell Proliferation and Scattering—To investigate the biological role of SHIP-1 in c-Met-mediated signaling, we overexpressed the Myc-tagged SHIP-1 gene in MDCK cells and isolated 19 different clones, each constitutively expressing distinct levels of an Myc-tagged SHIP-1. The two clones (clones 15 and 21) employed in these studies expressed equally high levels of SHIP-1 (Fig. 6A). Using these mutant cell lines together with the wild-type MDCK cells as control, we examined cell dissociation (scattering), cell proliferation, and branching tubulogenesis induced by HGF stimulation. Firstly, the scattering effect of HGF to both transfectants was indistinguishable from that of the wild type (Fig. 6B). Using these mutant cell lines together with the wild-type MDCK cells as control, we examined cell dissociation (scattering), cell proliferation, and branching tubulogenesis induced by HGF stimulation. Firstly, the scattering effect of HGF to both transfectants was indistinguishable from that of the wild type (Fig. 6B). Secondly, in agreement with data obtained from the fibroblast system (36), the overexpression of SHIP-1 showed no influence on [3H]thymidine incorporation, suggesting that overexpress-
sion of SHIP-1 did not affect cell growth of MDCK cells (Fig. 6C). Finally, we tested the ability of these cells to form tubules in semi-solid collagen. To our surprise, both transfectants that overexpressed Myc-tagged SHIP started to form branching tubules within 24 h after HGF treatment. Two days after HGF stimulation, transfectants formed long branching tubules (Fig. 7), whereas wild-type MDCK cells formed branching tubules only 6–7 days after HGF treatment (Fig. 7). Furthermore, in agreement with previous data (43), even 7 days after HGF treatment, about 70% of wild-type cells formed tubules; however, almost all transfectants formed tubules. In the absence of HGF, none of the transfectants formed any branching tubules throughout a time period of 2 weeks (data not shown). Taken together, these observations suggest that overexpression of SHIP clearly enhances HGF-mediated tubulogenesis.

Overexpression of a Mutant SHIP-1 Lacking Catalytic Activity in MDCK Cells Impaired HGF-mediated Branching Tubulogenesis—As shown above, SHIP-1 overexpression accelerated
tubulogenesis. This fact raised the question whether this phenotype results from enhanced phosphoinositol phosphatase activity or from displacement of protein binding to the receptor at the SHIP-1 binding site. To answer this question, we next generated a mutant SHIP-1 lacking catalytic activity by deleting amino acid residues 666–680 (44) using pcDNA3.1Myc-His, and we overexpressed this mutant in MDCK cells. Twelve different clones were isolated, and the two clones (clones 1 and 4) employed in this study expressed equally high levels of different clones were isolated, and the two clones (clones 1 and we overexpressed this mutant in MDCK cells. Twelve clones that overexpressed a mutant SHIP-1, however, failed to form branching tubules over 9 days (Fig. 8B), suggesting that the effects of SHIP-1 overexpression to accelerate tubulogenesis are due to the enhanced phosphoinositol phosphatase activity.

**DISCUSSION**

The results presented above can be summarized as follows. Firstly, we have identified SHIP as a novel binding partner of c-Met. Secondly, SHIP binds to tyrosine 1356 at the multifunctional docking site of c-Met. Thirdly, we showed here that this site, which contains two phosphotyrosine residues, binds in vitro to only one signaling molecule at a time. Fourthly, overexpression of SHIP drastically enhanced the tubulogenesis potency of c-Met without altering the c-Met-mediated cell scattering and cell proliferation. Cells that overexpressed a mutant SHIP-1 lacking catalytic activity, however, failed to form branching tubules in the presence of HGF, suggesting that the effects of SHIP-1 overexpression to accelerate tubulogenesis are due to the enhanced phosphoinositol phosphatase activity.

c-Met signaling is mediated by a multifunctional docking site comprising two phosphotyrosines arranged in tandem (20). Most tyrosine kinase receptors including c-Fms, c-Kit, and epidermal growth factor receptor autophosphorylate at multiple sites that bind several signaling molecules, suggesting that one receptor molecule is able to simultaneously associate with multiple signaling molecules. In addition, we showed here that the multifunctional docking site of c-Met binds to only one signaling molecule and that when several effector molecules are present simultaneously, one can observe in vitro the hierarchical binding of proteins, such as Grb2, Gab1, SHIP, and PI 3-kinase. Our competition assay reveals that PI 3-kinase did not compete with c-Met-Grb2 and c-Met-SHIP interactions; however, Grb2 competed with all of these interactions, suggesting that the binding order of these effector molecules is crucial for the c-Met-mediated multiple signal transduction. In vivo binding of full-length molecules, however, may be dependent upon their allosteric interactions. It is therefore also possible that multiple complexes of signaling molecules are assembled to the activated receptor. Furthermore, we have to take into account that the number and the subcellular distribution of each of the signaling molecules expressed in a given cell are different. For instance, when HGF-treated cells are grown in a three-dimensional collagen matrix, cells form tubules that have a lumen surrounded by well polarized epithelial cells, with a smooth basal surface in contact with the collagen matrix and an apical surface rich in microvilli that faces the lumen (11). The subcellular localization of c-Met and substrates differs in these cells from nonpolarized cells that are tested for cell scattering. For the cell-scattering assay, cells were incubated in liquid culture medium without collagen.

We show here that the SHIP-1 binding site, tyrosine 1356, is also a binding site for Grb2 with the YXXN motif. Does SHIP-1 regularly share the binding site with Grb2? In the case of c-Fms, tyrosine 696 and tyrosine 921 provide the binding sites for Grb2 with the YXXN motif, YNKYNI and YNYTLN1, respectively (31). Mutation of both sites, however, did not exert an influence on the SHIP-1-c-Fms association, indicating that none of these sites acts as a binding site for SHIP-1 (data not shown). These data suggest that the second or fourth flanking position is also important for binding. Interestingly, the SH2 domains of SHIP and Grb2 also share the binding site pYVNV of Shc (45), the sequence of which is identical to the pYVNV of c-Met, indicating that YVNV is a consensus sequence for the SHIP binding site (Table I). Interestingly, the point mutation of asparagine 1358 into histidine abolished the branching potential of c-Met (29).

A most striking observation of our studies regards the fact that overexpression of SHIP-1 drastically enhanced the HGF-mediated branching tubulogenesis without altering cell growth and cell scattering in response to HGF (Figs. 6 and 7). Here, both transfectants that overexpressed Myc-tagged SHIP started to form branching tubules within 24 h after the HGF treatment. SHIP plays a role in inositol phosphate and phosphatidylinositol phosphate metabolism (46). SHIP-1 displays 5-phosphatase activity specifically with both phosphatidylinositol 3,4,5-trisphosphate and inositol 1,3,4,5-tetraphosphate as substrate. Phee et al. (47) reported that enzymatic activity of SHIP is regulated by a plasma membrane localization and, as a result of this regulation, significant reduction in the cellular phosphatidylinositol 3,4,5-trisphosphate level was observed. Furthermore, a striking correlation was observed between phosphatidylinositol 3,4-bisphosphate production and tyrosine phosphorylation of SHIP-1, as well as its relocation to the cytoskeleton upon thrombin stimulation in human blood platelets (48). Here, phosphatidylinositol 3,4-bisphosphate may bind to pleckstrin homolog domains of still undefined enzymes, which may promote relocalization to the membrane and provide enzyme access to new lipid substrates or regulatory kinases. Indeed, the serine/threonine kinase B, also known as Akt has been shown to bind phosphatidylinositol 3,4-bisphos-
phate. In addition, the members of the protein kinase C family, PKCε and PKCθ, are activated by phosphorylating 3,4-bisphosphate (49). Interestingly, in the platelet system, Hartwig et al. (50) reported that phosphorylating 3,4-bisphosphate inhibits actin filament severing and capping by human gelsolin in vitro, suggesting that the product of SHIP-1 plays a role in the cytoskeleton rearrangement that is important for morphogenesis. On the other hand, Maroun et al. (51) reported that the ability of Gab1 to bind phosphorylating 3,4,5-trisphosphate is crucial for subcellular localization of Gab1 and for efficient morphogenesis mediated by c-Met. However, the same authors (43) reported that Gab1 phosphorylation per se is not sufficient to induce branching tubulogenesis and suggested that a Met-specific substrate, in addition to Gab1, is required for branching tubulogenesis. Recently, it has been reported that SHIP interacted with the protein inhibitor activated STAT1, PIAS1 (52). Another member of the STAT family, SHIP, has been reported that SHIP cDNA.

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