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hSHIP induces S-phase arrest and growth inhibition in cervical cancer HeLa cells

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Abstract

hSHIP, a human SH2-containing inositol-5-phosphatase, acts as a negative regulator of proliferation and survival in hematopoietic cells. Therefore, hSHIP may play a crucial role in suppression of cervical cancer HeLa cells. In this study, pcDNA3.1-hSHIP-GFP plasmid was constructed and transfected into HeLa cells with Lipofectamine2000, stably transfected HeLa cells were established and their responses were investigated by Flow cytometry, MTT, tumorigenicity in nude mice, RT-PCR and ELISA assays. The results showed that the expression of hSHIP significantly induced S-phase arrest, cell growth inhibition, and down-regulation of Akt1/2 mRNA and p-Akt in HeLa cells. Our study supports an important role for hSHIP in suppression of cervical cancer HeLa cells, which may prove to be a novel therapeutic option for non-hematopoietic cancers.

Keywords: SH2-containing inositol-5-phosphatase (SHIP); cell cycle; cell growth; PI3K/Akt; HeLa cells

Introduction

SHIP is a hematopoietic specific phosphoinositol 5′-phosphatase and was originally identified as a 145 kDa protein (Lioubin et al., 1996; Tu et al., 2001). SHIP functions, in part, by modifying a signaling pathway that is initiated by the activation of phosphatidylinositol 3-kinase (PI3K), a lipid kinase with pleiotropic effects (Aman et al., 1998; Hunter and Avalos, 1998). SHIP can associate with various adapter proteins, scaffold proteins, or receptors following the activation of hematopoietic cells (Kim et al., 1999; Liu et al., 1999). Formation of these complexes enables SHIP to hydrolyze the 5′-phosphate on PIP3 (Damen et al., 1996; Lioubin et al., 1996), thus preventing membrane recruitment and activation of pleckstrin homology domain-containing kinases, the effectors of PI3K signaling. SHIP is now recognized as an important negative regulator for cell activation in the mammalian hematopoietic cells (Liu et al., 1997; Zhang and Majerus, 1998).

Numerous studies have demonstrated that SHIP negatively regulates growth, cell cycle, and migration of hematopoietic cells. The hydrolysis of PIP3 by SHIP functions to limit the survival, activation, differentiation, and/or proliferation of hematopoietic cells (Liu et al., 1997). For example, overexpression of SHIP in a myeloid cell line inhibited cytokine-induced growth (Boer et al., 2001). SHIP mediates cell cycle arrest in mast cells *via* the F-gamma receptor IIB (FcγRIIB): a dose-dependent increase in the percentage of cells in S and G_2/M was observed following stimulation of FcγRIIB$^{+/+}$ by SHIP (Malbec et al., 2001). *SHIP*-deficient B cells have elevated and prolonged protein kinase B (PKB)/Akt and MAPK

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activation and fail to undergo FcγRIIb-mediated attenuation of B cell receptor (BCR)-induced proliferation (Phee et al., 2000). Mice with a disruption of the *SHIP* gene were found to be viable and fertile but failed to thrive, developing a myeloproliferative disorder characterized by extensive infiltration of myeloid cells in the lung. Additionally, marrow progenitor cells were hyperresponsive to hematopoietic growth factors in these mice (Helgason et al., 1998). This is of special interest since this myeloproliferative phenotype is similar to that of BCR/ABL transformation in mice (Daley et al., 1990; Elefanty et al., 1990; Heisterkamp et al., 1990; Kelliher et al., 1990).

PI3K/Akt constitutes an important pathway regulating the signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation and cell growth (Coffer and Woodgett, 1991; Hidalgo and Rowinsky, 2000; Osaki et al., 2004). Abnormal activation of this pathway such as PIK3C-alpha (*PIK3C-α*) mRNA amplification, inappropriate activation of Akt, and Akt protein overexpression (Samuels et al., 2004; Shukla et al., 2005) occurs in a wide variety of cancers, including cervical cancer (Zhang et al., 2008), oophoroma (Levine et al., 2005), breast cancer (Levine et al., 2005), spongioblastoma (Knobbe et al., 2005), cancer of the endometrium (Oda et al., 2005), and colonic cancer (Wang et al., 2007). Selective targeting of tumor cells by major members of the PI3K/Akt pathway opens up new perspectives for tumor therapy.

In the previous studies, there were several reports of SHIP in non-hematopoietic cells. Mancini et al. (2002) reported that SHIP is implicated in the control of cell-cell junction and the induction of the dissociation and dispersion of MDCK cells. Xing and Hamaguchi (2007) found that the overexpression of SHIP does not affect matrix metalloproteinase 2 (MMP2) secretion in both *Src*-transformed 3Y1 cells and wild type 3Y1 cells, but can induce MMP9 secretion and also negatively regulates cell migration and invasion in transformed cells. In this study, we found, for the first time, the expression of hSHIP in HeLa cells induced S-phase arrest, cell growth inhibition and down-regulation of Akt1/2 mRNA and p-Akt.

Materials and methods

Vector constructs

Full-length cDNA of hSHIP was obtained by RT-PCR using the following primers: forward, 5′-ATGTAAGCTT GCCACCATGGTCC CCTGCTGGAAC-3′ and reverse, 5′-ATTGGATCCCTGCATGGCAGTCCTGCCTAG-3′. The forward and reverse primers contained the restriction sites *Hin*d III and *Bam*H I, respectively (enzyme recognition sites are underlined). The PCR products were purified, digested and inserted into pcDNA3.1-GFP. The recombinant plasmid was transformed into *Eschericia coli* DH5α cells. The positive recombinant plasmid was identified by dual digestion with *Hin*d III and *Bam*H I and subsequent sequencing by Shanghai Invitrogen Co. Ltd. (Shanghai, China).

Cell culture and establishment of stable cell lines

HeLa cells (maintained in the laboratory) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (Gibco, USA) in 5% CO_2 at 37°C. HeLa cell clones expressing hSHIP-GFP (HeLa/hSHIP-GFP) and GFP (HeLa/GFP, control) were established in our laboratory. Briefly, pcDNA3.1-hSHIP-GFP and pcDNA3.1-GFP (control) were transfected into HeLa cells by Lipofectamine2000 (Invitrogen, USA), respectively. Stably transfected clones were selected in medium containing 1,000 μg/mL of G418 (Amresco, USA). The G418-resistant clones were isolated, transferred and subcultured. Stable cell clones were verified by Western blot. Once established, clones were maintained in DMEM medium containing 500 μg/mL of G418.

Western blot

Cell lysates (HeLa/hSHIP-GFP and HeLa/GFP) were used for Western blot analysis (Xing and Hamaguchi, 2007). The anti-GFP monoclonal antibody (Beyotime, China) was used at 1:1,000 dilution. Monoclonal anti-β-actin (Santa cruz, USA) was used at 1:5,000 dilution.

Cell cycle analysis

HeLa/hSHIP-GFP and HeLa/GFP cells were harvested and fixed in ice-cold 70% ethanol and stored at –20°C overnight. The fixed cells were washed with cold phosphate buffer solution (PBS) and incubated at 37°C for 30 min in 0.5 mL PBS containing 10 μg/mL propidium iodine (Sigma, USA) and 5 μg/mL RNaseA (Sigma). DNA content was determined by Flow cytometry analysis.

MTT assay

MTT assay was performed as described previously (Kim et al., 2003). Briefly, cells were washed with PBS, counted, and diluted to 10,000 cells/mL. Cells (1,000 cells/well) were plated in 96 well plates. After 24 h, 48 h, 72 h, and 96 h of incubation in 5% CO_2 at 37°C, MTT (Sigma) labeling reagent (10 μL) was added, followed by the addition of 100 μL of solubilization solution 4h later. The production of blue formazan produced by viable cells was measured on a microplate reader at an absorbance of 570 nm. Experiments were performed in triplicate.

Tumorigenicity assay in nude mice

We randomly divided twelve 5-week-old female nude mice into two experimental groups: a HeLa/GFP group and a HeLa/hSHIP-GFP group. The two different cell lines were suspended in 0.1 mL PBS (containing 2×10^6 cells) and were injected subcutaneously into the back of the mice. After 6 days, tumor dimensions were measured using calipers every two days and tumor volume was calculated using the following formula: $1/6\pi \times a \times b^2$, where a is the tumor length, b is the width.

Semi-quantitative RT-PCR

Total RNA was extracted from cultured cells (HeLa/hSHIP-GFP and HeLa/GFP) using TRIzol® Reagent (Invitrogen) and RT-PCR was performed according to the manufacturer's instructions (MBI Fermentas, USA). The gene-specific primers used were as follows: for human Akt1 forward: 5′-GGCGAGCTGTTCTTCCACCT-3′, Akt1 reverse: 5′-ATTGTCCTCCAGCACCTCGG-3′ (yielding a 311 bp product); Akt2 forward: 5′-CTGAGGTGCTGGA GGACAAT-3′, Akt2 reverse: 5′-GAGCTTCTTCTGGA CCACGT-3′ (yielding a 276 bp product). PCR products were separated on a 1.5% agarose gel. *β-actin* (forward: 5′-AGCGAGCATCCCCCAAAGTT-3′, reverse: 5′-GGGC ACGAAGGCTCATCATT-3′; yielding a 268 bp product) was used as an internal control. The gels were analyzed with Quantity One software.

ELISA

Phosphorylation of Akt at serine 473 (p-Akt[Ser473]) of cells (HeLa/hSHIP-GFP, HeLa/GFP) was assessed with ELISA kit (Cat. #KHO0111, Biosource International, Inc., Camarillo, CA, USA) according to the manufacturer's instructions.

Results

Expression of hSHIP-GFP and GFP proteins detected by Western blot

Stable transfectants were selected from HeLa cells by G418. Western blot analysis verified that stable G418-resistant colonies with transfection of pcDNA3.1- hSHIP-GFP expressed 171 kDa hSHIP-GFP fusion protein and stable G418-resistant colonies with transfection of pcDNA3.1-GFP expressed 27 kDa GFP protein (Fig. 1). The molecular masses of hSHIP-GFP and GFP were consistent with the predicted molecular masses, showing that stable cell lines (HeLa/hSHIP-GFP, HeLa/GFP) were established.

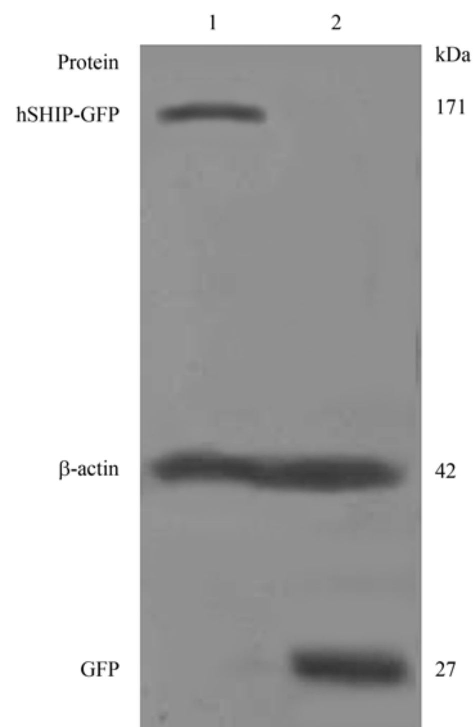

Fig. 1. The expression of GFP and hSHIP-GFP proteins was detected by Western blot. 1: HeLa/hSHIP-GFP cells; 2: Hela/GFP cells. Cell lysates (HeLa/hSHIP-GFP, HeLa/GFP) were separated by SDS-PAGE, transferred to nitrocellulose membranes and detected with an anti-GFP antibody. The level of β-actin was used as a control for equal loading of protein.

hSHIP-induced S-phase arrest

In order to investigate whether hSHIP had the ability to inhibit the cell cycle in HeLa cells, cell cycle analysis was performed on HeLa/hSHIP-GFP and HeLa/GFP cells. Flow cytometry analysis showed that the expression of hSHIP remarkably increased the proportion of HeLa cells in S-phase (from (27.30 ± 4.50)% to (47.20 ± 7.78)%, $P < 0.05$) and decreased the proportion of cells in G_2/M phase (from (14.45 ± 1.91)% to (2.60 ± 0.71)%, $P < 0.05$) (Fig. 2). These data showed that hSHIP significantly induced S-phase arrest of HeLa cells.

hSHIP-mediated growth suppression

After plating the cells, the MTT assay was then performed. Cell growth was determined at 24 h, 48 h, 72 h, and 96 h. The growth inhibition curves are shown in Fig. 3A. Compared with HeLa/GFP cells, the viability of HeLa/hSHIP-GFP cells was reduced by 78.12% at 24 h, 75.08% at 48 h, 78.12% at 72 h, 80.19% at 96 h ($P < 0.05$). Moreover, tumors formed in nude mice 6 days after the injection of HeLa/GFP cells, while tumors formed from HeLa/hSHIP-GFP cells after 10 days and grew more slowly. The tumor volume generated from HeLa/hSHIP-GFP cells was smaller than that from HeLa/GFP cells (510.75 ± 80.01 mm^3 *vs.* 136.73 ± 33.07 mm^3 after 28 days, $P < 0.01$, Fig. 3B). These data indicated that the exogenous hSHIP had a negative effect on HeLa tumor growth.

Down-regulation of Akt1/2 mRNA expression and phosphorylation of Akt by hSHIP

To investigate whether hSHIP modulates the expression of Akt in HeLa cells, semi-quantitative RT-PCR analysis was used to determine the levels of Akt1/2 mRNA in the two different cell lines. The percentage of *Akt1* mRNA declined to (30.52 ± 4.8)% ($P < 0.05$) while the *Akt2* mRNA declined to (31.83 ± 3.52)% ($P < 0.05$) in HeLa/hSHIP-GFP cells compared with the control (Fig. 4).

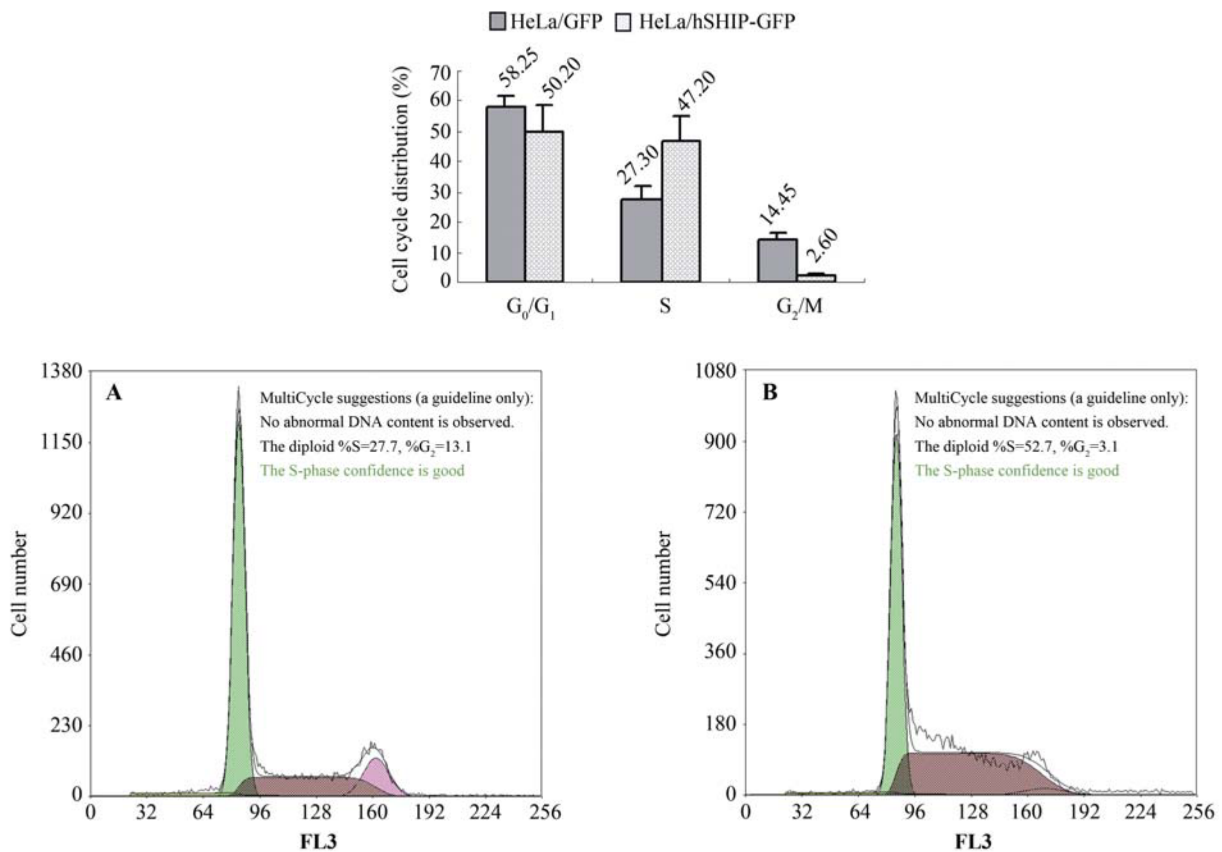

Fig. 2. hSHIP-induced S arrest in HeLa cells. **A:** HeLa/GFP cells (control); **B:** HeLa/hSHIP-GFP cells. Cells were cultured in complete medium and were collected, washed with PBS, digested with RNaseA, stained with PI, and analyzed by Flow cytometry. The values represent the number of cells in each phase of the cell cycle as a percentage (%) of total cells. Data represent mean ± SD of three independent experiments.

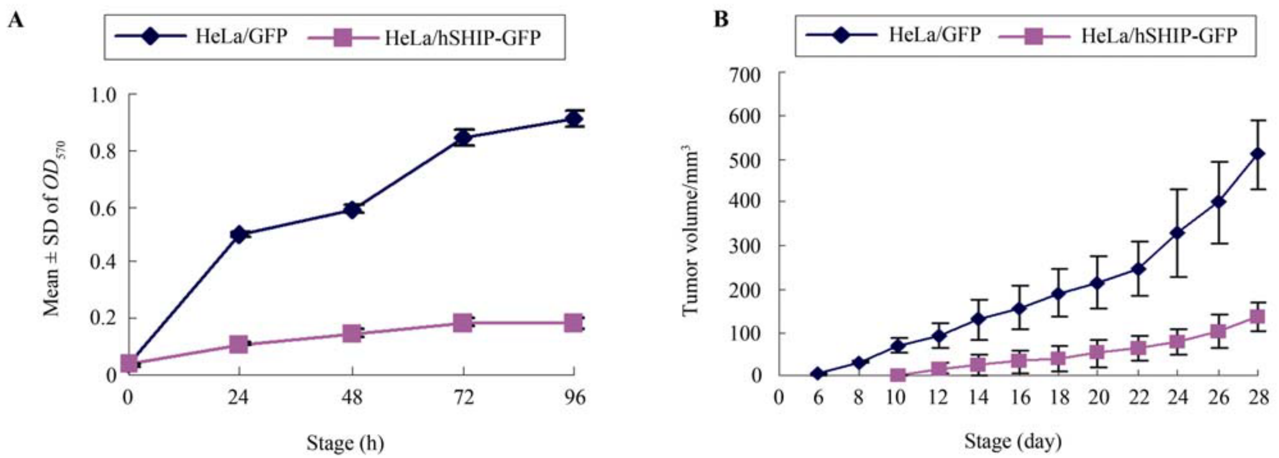

Fig. 3. hSHIP mediated growth suppression in HeLa cells. **A:** hSHIP expression causing *in vitro* inhibition of growth of HeLa cells by MTT. **B:** hSHIP expression causing *in vivo* inhibition of growth and tumorigenicity in nude mice.

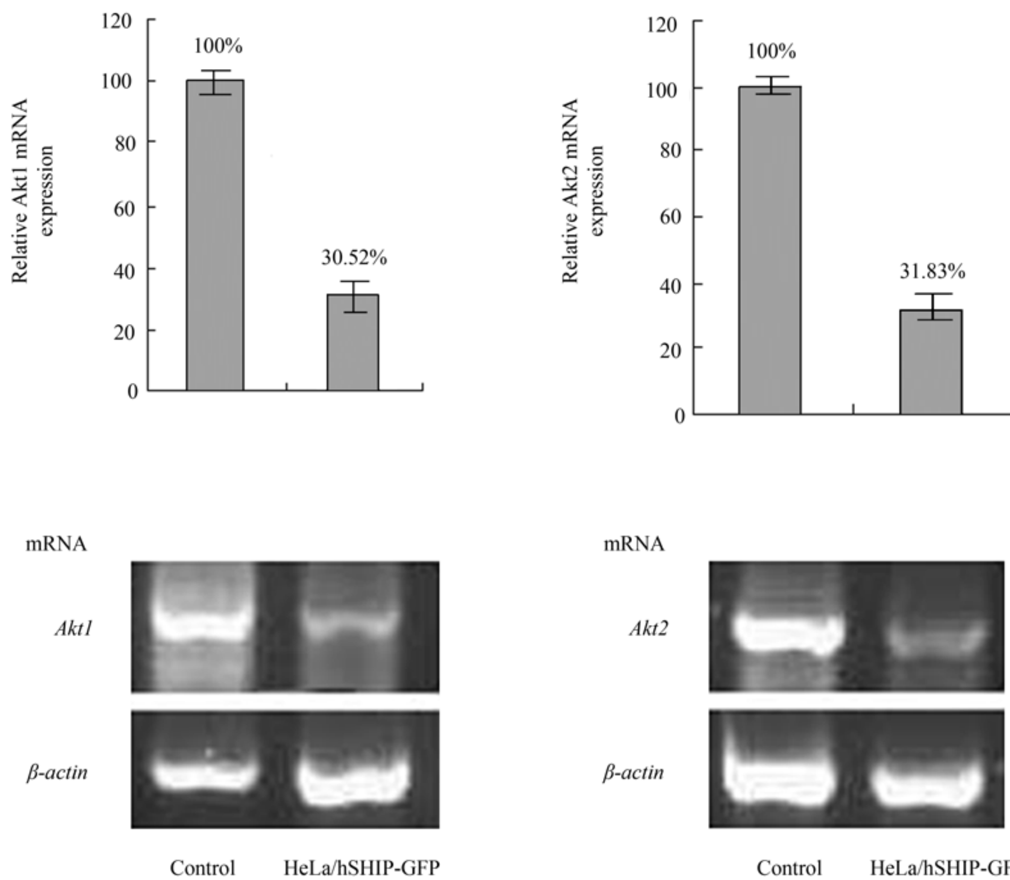

Fig. 4. Expression of Akt1/2 mRNA in HeLa/hSHIP-GFP cells was assessed by semi-quantitative RT-PCR. Cells were harvested for RNA isolation. RT-PCR products were electrophoresed on a 1.5% agarose gel. The level of *β-actin* was used as an internal control.

Furthermore, hSHIP expression in HeLa cells induced the down-regulation of p-Akt (Ser473). ELISA results showed that the relative percentage of p-Akt was decreased from (100 ± 9.87)% to (29.17 ± 5.83)% ($P < 0.05$) in HeLa/hSHIP-GFP cells compared with the control (Fig. 5).

Discussion

Akt (PKB) is a central protein of the PI3K/Akt signaling pathway and its activation stimulates cell cycle progression, survival, apoptosis, and migration. Akt is a serine-threonine kinase, downstream of PI3K, which has three family members: Akt1, Akt2 and Akt3 (Altomare and Testa, 2005). Activated phosphoinositide-dependent kinase-1 (PDK1) phosphorylates Akt at Thr308, thereby activating its serine-threonine kinase activity. Once phosphorylated at Thr308, further activation occurs *via* phosphoinositide-dependent kinase-2 (PDK2) phosphorylation at Ser473. In this study, we found that hSHIP can significantly down-regulate the expression of Akt mRNA (Fig. 4) and phosphorylation of Akt (Ser473) in HeLa cells (Fig. 5). It is well documented that SHIP can inhibit Akt activation through dephosphorylation of PIP3, the product of PI3K. PIP3 is required for translocation of Akt to the cell membrane where Akt is phosphorylated and activated by upstream kinases.

The cell cycle progresses sequentially through G_1, DNA synthesis (S), G_2, and mitosis (M) phase, which regulates DNA replication and chromosomal segregation into the daughter cells. Cell cycle progression is precisely regulated by a series of cyclin-dependent kinases (CDKs), whose activities are strongly dependent on their association with the cyclin subunits (Pines, 1995) and a kind of cyclin-dependent kinase inhibitor. HeLa/hSHIP-GFP cells were delayed at the S-phase compared with the control (Fig. 2). It is possible that hSHIP induced a decrease in p-Akt which promotes the breakdown of cyclins and removal of CKIs-mediated inhibition.

The MTT assay and tumorigenicity assay in nude mice (Fig. 3) showed that hSHIP significantly inhibits the growth of HeLa cells. Cell growth requires both proliferation signals and survival signals. We postulated that hSHIP suppresses cell growth through the negative regulation of the cell cycle.

Our study has demonstrated that hSHIP induced S-phase

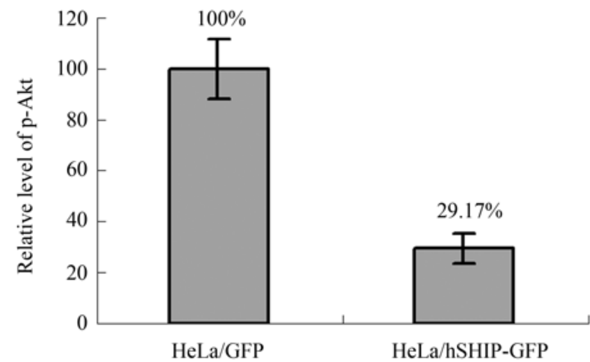

Fig. 5. The phosphorylation of Akt analyzed by ELISA. Cells (HeLa/hSHIP-GFP, HeLa/GFP) were lysed and assayed for p-Akt (Ser473). hSHIP induced reduction of level of phosphorylation at Ser473.

arrest, cell growth inhibition and the down-regulation of Akt and p-Akt in HeLa cells. These results contribute to the further study of SHIP-induced cell cycle arrest and growth inhibition in nonhematopoietic cancers and to the precise mechanisms involved in PI3K/Akt-dependent, SHIP-mediated S-phase arrest and cell growth inhibition.

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References

Lioubin, M.N., Algate, P.A., Tsai, S., Carlberg, K., Aebersold, A., and Rohrschneider, L.R. (1996). p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity. Genes Dev. **10:** 1084–1095.

Tu, Z., Ninos, J.M., Ma, Z., Wang, J.W., Lemos, M.P., Desponts, C., Ghansah, T., Howson, J.M., and Kerr, W.G. (2001). Embryonic and hematopoietic stem cells express a novel SH2-containing inositol 5′-phosphatase isoform that partners with the Grb2 adapter protein. Blood **98:** 2028–2038.

Aman, M.J., Lamkin, T.D., Okada, H., Kurosaki, T., and Ravichandran, K.S. (1998). The inositol phosphatase SHIP inhibits Akt/PKB activation in B cells. J. Biol. Chem. **273:** 33922–33928.

Hunter, M.G., and Avalos, B.R. (1998). Phosphatidylinositol 3'-kinase and SH2-containing inositol phosphatase (SHIP) are recruited by distinct positive and negative growth-regulatory domains in the granulocyte colony-stimulating factor receptor. J. Immunol. **160:** 4979–4987.

Kim, C.H., Hangoc, G., Cooper, S., Helgason, C.D., Yew, S.,

Humphries, R.K., Krystal, G., and Broxmeyer, H.E. (1999). Altered responsiveness to chemokines due to targeted disruption of SHIP. J. Clin. Invest. **104:** 1751–1759.

Liu, Q., Sasaki, T., Kozieradzki, I., Wakeham, A., Itie, A., Dumont, D.J., and Penninger, J.M. (1999). SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Genes Dev. **13:** 786–791.

Damen, J.E., Liu, L., Rosten, P., Humphries, R.K., Jefferson, A.B., Majerus, P.W., and Krystal, G. (1996). The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. Proc. Natl. Acad. Sci. USA **93:** 1689–1693.

Zhang, X., and Majerus, P.W. (1998). Phosphatidylinositol signalling reactions. Semin. Cell Dev. Biol. **9:** 153–160.

Liu, L., Damen, J.E., Ware, M., Hughes, M., and Krystal, G. (1997). SHIP, a new player in cytokine-induced signalling. Leukemia **11:** 181–184.

Boer, A.K., Drayer, A.L., and Vellenga, E. (2001). Effects of overexpression of the SH2-containing inositol phosphatase SHIP on proliferation and apoptosis of erythroid AS-E2 cells. Leukemia **15:** 1750–1757.

Malbec, O., Schmitt, C., Bruhns, P., Krystal, G., Fridman, W.H., and Daeron, M. (2001). Src homology 2 domain-containing inositol 5-phosphatase 1 mediates cell cycle arrest by FcgammaRIIB. J. Biol. Chem. **276:** 30381–30391.

Phee, H., Jacob, A., and Coggeshall, K.M. (2000). Enzymatic activity of the Src homology 2 domain-containing inositol phosphatase is regulated by a plasma membrane location. J. Biol. Chem. **275:** 19090–19097.

Helgason, C.D., Damen, J.E., Rosten, P., Grewal, R., Sorensen, P., Chappel, S.M., Borowski, A., Jirik, F., Krystal, G., and Humphries, R.K. (1998). Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. Genes Dev. **12:** 1610–1620.

Daley, G.Q., Van Etten, R.A., and Baltimore, D. (1990). Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science **247:** 824–830.

Elefanty, A.G., Hariharan, I.K., and Cory, S. (1990). bcr-abl, the hallmark of chronic myeloid leukaemia in man, induces multiple haemopoietic neoplasms in mice. EMBO J. **9:** 1069–1078.

Heisterkamp, N., Jenster, G., ten Hoeve, J., Zovich, D., Pattengale, P.K., and Groffen, J. (1990). Acute leukaemia in bcr/abl transgenic mice. Nature **344:** 251–253.

Kelliher, M.A., McLaughlin, J., Witte, O.N., and Rosenberg, N. (1990). Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. Proc. Natl. Acad. Sci. USA **87:** 6649–6653.

Coffer, P.J., and Woodgett, J.R. (1991). Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. Eur. J. Biochem. **201:** 475–481.

Hidalgo, M., and Rowinsky, E.K. (2000). The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene **19:** 6680–6686.

Osaki, M., Oshimura, M., and Ito, H. (2004). PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis **9:** 667–676.

Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S.M., Riggins, G.J., Willson, J.K., Markowitz, S., Kinzler, K.W., Vogelstein, B., and Velculescu, V.E. (2004). High frequency of mutations of the *PIK3CA* gene in human cancers. Science **304:** 554.

Shukla, S., Maclennan, G.T., Marengo, S.R., Resnick, M.I., and Gupta, S. (2005). Constitutive activation of P I3 K-Akt and NF-kappaB during prostate cancer progression in autochthonous transgenic mouse model. Prostate **64:** 224–239.

Zhang, X.Y., Zhang, H.Y., Zhang, P.N., Lu, X., and Sun, H. (2008). Elevated phosphatidylinositol 3-kinase activation and its clinicopathological significance in cervical cancer. Eur. J. Obstet. Gynecol. Reprod. Biol. **139:** 237–244.

Levine, D.A., Bogomolniy, F., Yee, C.J., Lash, A., Barakat, R.R., Borgen, P.I., and Boyd, J. (2005). Frequent mutation of the *PIK3CA* gene in ovarian and breast cancers. Clin. Cancer Res. **11:** 2875–2878.

Knobbe, C.B., Trampe-Kieslich, A., and Reifenberger, G. (2005). Genetic alteration and expression of the phosphoinositol-3-kinase/Akt pathway genes *PIK3CA* and *PIKE* in human glioblastomas. Neuropathol. Appl. Neurobiol. **31:** 486–490.

Oda, K., Stokoe, D., Taketani, Y., and McCormick, F. (2005). High frequency of coexistent mutations of *PIK3CA* and *PTEN* genes in endometrial carcinoma. Cancer Res. **65:** 10669–10673.

Wang, J., Kuropatwinski, K., Hauser, J., Rossi, M.R., Zhou, Y., Conway, A., Kan, J.L., Gibson, N.W., Willson, J.K., Cowell, J.K., and Brattain, M.G. (2007). Colon carcinoma cells harboring *PIK3CA* mutations display resistance to growth factor deprivation induced apoptosis. Mol. Cancer Ther. **6:** 1143–1150.

Mancini, A., Koch, A., Wilms, R., and Tamura, T. (2002). The SH2-containing inositol 5-phosphatase (SHIP)-1 is implicated in the control of cell-cell junction and induces dissociation and dispersion of MDCK cells. Oncogene **21:** 1477–1484.

Xing, W., and Hamaguchi, M. (2007). Effects of SHIP-1 on MMP2 secretion and invasion of SR3Y1 cells. J. Genet. Genomics **34:** 285–293.

Kim, S., Kang, J., Hu, W., Evers, B.M., and Chung, D.H. (2003). Geldanamycin decreases Raf-1 and Akt levels and induces apoptosis in neuroblastomas. Int. J. Cancer **103:** 352–359.

Altomare, D.A., and Testa, J.R. (2005). Perturbations of the AKT signaling pathway in human cancer. Oncogene **24:** 7455–7464.

Pines, J. (1995). Cyclins and cyclin-dependent kinases: theme and variations. Adv. Cancer Res. **66:** 181–212.