Published in final edited form as:

Biochem Biophys Res Commun. 2006 December 29; 351(4): 979–985.

HSP70 binds to SHP2 and has effects on the SHP2 related EGFR/GAB1 signaling pathway

Jae Cheal Yoo and Michael J. Hayman

From the Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, New York, 11794-5222

Abstract

The Src homology phosphotyrosyl phosphatase, SHP2, is a positive effector of EGFR signaling. However, the molecular mechanism and biological functions of SHP2 regulation are still not completely known. To better understand the cellular processes in which SHP2 participates, we carried out mass spectrometry to find SHP2 binding proteins. FLAG-SHP2 complexes were isolated by affinity purification, and associated proteins were identified by in-gel trypsin digestion followed by LC/MS/MS mass spectrometry. Among the identified proteins, we focus in this report on the heat shock protein 70 (HSP70). Physical interactions of SHP2 with HSP70 were confirmed *in vivo*. Further experiments demonstrate that EGF does not activate binding of SHP2 with HSP70 rather the binding appears to be constitutive. However, the formation of an HSP70/SHP2 complex affected the binding of SHP2 with EGFR and (or) GAB1. These data suggest that binding of HSP70 with SHP2 regulates to some extent the EGF signaling pathway. In addition, immunostaining experiments indicated that SHP2 and HSP70 co-localized in the cell membrane region after EGF treatment. Our findings propose a possible involvement of HSP70 in the regulation of EGF signaling pathway by SHP2.

INTRODUCTION

SHP2 is a phosphatase that plays a pivotal role in the biological responses to growth factors, hormones, cytokines, and cell adhesion molecules. Many different functions of SHP2 have been reported. SHP2 regulates the recruitment of p120Ras-GAP to RTKs [1,2], controls the recruitment of c-Src kinase (Csk) [3,4] and the tyrosyl phosphorylation and activity of Sprouty proteins, a family of Ras inhibitors [5]. Similarly, SHP-2 can play a role in PI3K signaling from activated growth factor receptors [6,7].

HSP70 is a highly conserved protein and is among the most abundant molecular chaperone sub-types. HSP70 is a molecular chaperone protein activated by various cellular stresses including heat shock, oxygen-free radicals, inflammation, oncogenes, and proto-oncogenes [8]. Furthermore, HSP70 is known to regulate the catalytic activity, conformational maturation of binding proteins, and contributes to cell growth and survival [9-11].

Previously, we were able to develop an efficient trapping mutant of SHP2 by introducing D425A/C459S double mutations (SHP2DM) [12]. In this study we have carried out a mass spectrometry analysis to identify SHP2 binding proteins that associate with N-terminal FLAG tagging-SHP2WT and -SHP2DM (FSHP2WT and FSHP2DM).

MATERIALS AND METHODS

Cells and Plasmid construction

The cell type used in this study was COS-1 cells. Construction of plasmids that express SHP2WT or DM-SHP2DM was described previously [13]. pHSP70 was a gift from Dr. Morimoto (Northwestern University, Illinois, USA). For purification of N-terminal FLAG-tagged SHP2WT or SHP2DM proteins, corresponding cDNA was cut out from the pMT2 vector with *EcoRI* and then inserted in-frame into pCMV-Tag 2A.

Isolation of FLAG-SHP2 Complexes

FLAG-SHP2WT or -SHP2DM transfected cells were cultured 36 hours, and then treated with 100ng/ml EGF for 10 minutes. An equal number of cells transfected with vector alone were cultured as a negative control. Cells were disrupted in RIPA buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1% triton X-100, and 0.1% SDS) containing a mixture of protease inhibitors. Clarified cellular lysates were incubated with FLAG M2 agarose gel for 3 hours at 4°C. The resin was collected by centrifugation. FLAG-SHP2 complexes were eluted from the resin by incubation with FLAG peptide in TBS and acetone precipitated.

Mass Spectrometry

Visible protein bands were excised from the colloidal blue-stained gel and chopped into 1-mm³ pieces. LC/MS/MS experiments were performed by the Proteomics Center at the Stony Brook University on an API Qstar Pulsar i quadrupole-TOF LC/MS/MS mass spectrometer. Protein identification was done by the ProID software to search the Swissprot protein sequence database.

Cell transfection and preparation of lysates

Cells were transfected with various constructs by using the FuGene transfection reagent, incubated for 36 hours, serum starved for 12 hours, and then stimulated with 100 ng/ml of EGF. After being washed twice with PBS, cells were lysed in RIPA buffer with protease inhibitors for 30 minutes on ice and centrifuged at 5,000g for 30 minutes at 4°C. The supernatant was analyzed as described.

Immunoprecipitation and western blot

The supernatants were incubated with the indicated antibodies for 3 hours, at 4°C with an additional incubation for 2 hours after addition of protein A- or G-Sepharose beads. Immunocomplexes captured on Sepharose beads were washed three times with RIPA buffer, eluted by being boiled with SDS gel-loading buffer. The immunoprecipitates were analyzed by western blot. The western blot analysis was carried out as described previously [14].

Immunocytochemistry Analysis

Cells were rinsed with PBS, fixed in 3.5% paraformaldehyde in PBS for 10 minutes at room temperature, permeabilized with 0.1% triton X-100 for 20 minutes, and incubated with the primary antibodies in 3% BSA for 1 hour. After a brief washing, the cells were incubated with the secondary antibodies conjugated with FITC/TRITC, mounted in a mounting solution with DAPI, and observed with a fluorescence microscope.

RESULTS

Purification of SHP2 Complexes

As discussed above previous experiments have identified both substrates and binding partners of SHP2. However, SHP2's function in various signal pathways is still not clear. Therefore we decide to try and find and analyze additional SHP2 binding proteins.

To establish a reliable system for affinity purification of SHP2 complexes, *FSHP2WT* and *FSHP2DM* were subcloned into an expression vector (see Materials and Methods). To identify binding partners of SHP2, cells transfected with these constructs were treated with EGF for 10 minutes. FLAG-SHP2 proteins were immunoprecipitated with anti-FLAG M2 affinity gel.

Identification of HSP70 in SHP2 Complexes

A prominent band of ~70 kDa found in the *FSHP2DM* transfected sample was subjected to further analysis (Data not shown). This band was excised from the gel and digested with trypsin. The resulting tryptic peptides were subjected to Mass Spectrometry analysis. SEQUEST was used to match MS with proteins in the Swissprot protein sequence database. Based upon these analyses, the p70 band contained both SHP2 and HSP70 respectively (Data not shown).

HSP70 Is Associated with SHP2

We wanted to confirm that the HSP70 protein could associate with SHP2. Co-immunoprecipitation (Co-IP) experiments were carried out to examine physical interactions between the SHP2 and HSP70 proteins. Cells that had been transfected transiently with the *FSHP2WT* or *FSHP2DM* were untreated or treated for 10 minutes with EGF. These cells were lysed with RIPA buffer and subjected to IP using anti-HSP70 or -FLAG antibodies. Either antibody against the SHP2 or HSP70 proteins was then used for detection of proteins present in the immunoprecipitates. Supplementary Fig 1 (Sup Fig 1) shows that the antibodies against SHP2 and HSP70 could immunoprecipitate the respective proteins. Re-probing of the filters demonstrated the interaction of both *FSHP2WT* and *FSHP2DM* with endogenous HSP70 proteins; regardless of whether HSP70 was immune precipitated or SHP2 was immune precipitated. Furthermore, binding of SHP2 with HSP70 did not change following EGF treatment for 10 minutes (Sup Fig 1). Moreover, as shown below, endogenous SHP2 binds with endogenous HSP70 in non-transfected cells (Fig 4B). These results indicate that SHP2 interacts with HSP70 *in vivo*. But this interaction is not EGF-dependent neither does it increase in the trapping mutant transfected cell indicating that HSP70 is not a substrate of SHP2 but is a binding partner.

HSP70 Binding with SHP2 is not Dependent on EGF Treatment

Because the ~70 kDa band in *SHP2DM* transfected cells was so strong with 4G10 antibody following EGF treatment (Sup Fig 2) but not in *SHP2WT* expressing cells, we decided to determine if SHP2 binds with HSP70 in a time dependent manner. We were concerned that the tagging of the SHP2 constructs may affect the result, so non-tagged SHP2 constructs were used. *SHP2WT* or *SHP2DM* with/without HSP70 expressed in cells were immunoprecipitated with the SHP2 antiserum after EGF treatment for the indicated times. Again SHP2 co-precipitated with the HSP70 protein (Fig 1) and there was no change of binding capacity of SHP2 with HSP70 after EGF treatment for the indicated times (Fig 1). In addition, as shown above, SHP2 binds with HSP70 even in cells not treated with EGF (Fig 1). These data confirm that this interaction is not dependent on EGF treatment. Furthermore, these results also indicate that HSP70 is not a substrate of SHP2 in EGF signaling pathway. Consistent with this conclusion, there are no reports of HSP70 being phosphorylated on tyrosine.

HSP70 Regulates SHP2 Binding with EGFR

A comparison of *SHP2WT* alone with *SHP2WT/HSP70* transfected cell lysates indicated that the tyrosine phosphorylation of SHP2 binding proteins of approximately 180 kDa almost disappeared when HSP70 was co-transfected (Fig 1). Interestingly, this difference was barely seen in cells expressing the *SHP2DM* mutant. This would argue that SHP2 has to be catalytically active in order for HSP70 to exert this effect.

Previously it has been shown, that SHP2 binds to the EGFR [1], which is of an approximate molecular weight of 180,000. Thus the SHP2 binding protein whose tyrosine phosphorylation is affected (Fig 1) could well be EGFR. We thought that HSP70 may affect EGFRSHP2 binding. To test this hypothesis, we assayed SHP2-EGFR binding when *SHP2WT* only, *HSP70/SHP2WT* or *HSP70/SHP2DM* were expressed in cells.

Endogenous EGFR expression levels were similar in *SHPWT* only, *SHP2WT/HSP70*, *SHP2DM/HSP70* transfected, and non-transfected cell lysates (Fig 2A). Moreover, some SHP2 binding proteins were highly phosphorylated after EGF treatment in *SHP2WT* only or *SHP2DM/HSP70* transfected cell lysates (Fig 2A). These cell lysates were immunoprecipitated with anti-SHP2 antiserum. The binding level of SHP2 with HSP70 was barely changed after EGF treatment (Fig 2B). Interestingly, SHP2 only weakly co-immunoprecipitated with EGFR in the *SHP2WT/HSP70* co-transfected cell lysates even though they were treated with EGF (Fig 2B). Moreover, the *SHP2DM* mutant strongly binds with EGFR even though HSP70 was highly expressed (Fig 2B). These results indicate that increased expression of HSP70 does not affect the binding of the catalytically-dead “substrate-trapping” SHP2 mutant (*SHP2DM*) but it does appear to influence the interaction of wild type SHP2 with EGFR after EGF treatment in cells.

HSP70 Regulate SHP2 Binding with GAB1

There are reports that SHP2 attenuates PI3K activation in response to EGF by dephosphorylation of the p85 binding sites on GAB1 [7]. So, we tested whether binding of SHP2 with HSP70 influences the interaction of GAB1 with its binding proteins. To test this possibility the binding levels of SHP2 with GAB1 were checked in *HSP70*, *HSP70/SHP2WT* or *HSP70/SHP2DM* expressing cells. GAB1 expression levels were similar in all *FLAGGAB1 (FGAB1)* only, *SHPWT/FGAB1*, and *SHP2WT/HSP70/FGAB1*, transfected cell lysates (Sup Fig 3A). Expressions of SHP2 and HSP70 proteins in their transfected cell lysates are higher than non-transfected cell lysates showing successful transfection (Sup Fig 3A).

The cell lysates were immunoprecipitated with anti-SHP2 or FLAG antibodies to bring down the GAB1 protein (Sup Fig 3B). In the IP with anti-SHP2 antiserum, the amount of HSP70 binding to SHP2 was not changed but as expected GAB1 binding with SHP2 was increased following EGF treatment (Sup Fig 3B, left panel). But in *SHP2WT/HSP70* transfected cell lysates, the binding of GAB1 to SHP2 remained weak after EGF treatment when compared with *SHP2WT* only transfected cell lysates (Sup Fig 3B, left panel). This indicates that the co-expression of *SHP2WT* and HSP70 affects the association of GAB1 with SHP2. This effect appears to be dependent on the catalytic activity of SHP2 since it was not seen in cells transfected with the catalytically inert SHP2 mutant as well as HSP70 (data not shown).

Following IP with anti-FLAG antibody to precipitate the GAB1 protein it was noted that, as expected, the p85 subunit of PI3K and SHP2 association with GAB1 was increased following EGF treatment (Sup Fig 3B, right panel). In this case the co-expression of HSP70 had no effect on the association of PI3K with GAB1, whereas there was again a small reduction in the association of SHP2 with GAB1. Next, we tried to confirm that SHP2 binding with GAB1 is affected by increased expression of HSP70 and (or) EGF treatment in a time dependent manner.

When *SHP2WT/FGAB1* with (without) *HSP70* transfected cell lysates were immunoprecipitated with the SHP2 antiserum after EGF treatment for the indicated times, the expression level of SHP2 and level of HSP70 binding with SHP2 is not changed (Fig. 3, top panel), regardless of whether endogenous or transfected HSP70 is measured. This again indicates that the association of HSP70 and SHP2 is constitutive and is not affected by EGF. In contrast, in HSP70 transfected cell lysates, GAB1 and phosphorylated GAB1 binding with SHP2 decreases dramatically over time after EGF treatment (Fig. 3, top panel). This may mean that the increased levels of HSP70 somehow regulate binding of SHP2 with GAB1.

SHP2WT/FGAB1 with (without) *HSP70* transfected cell lysates were immunoprecipitated with the FLAG antibodies after EGF treatment for the indicated times, the expression level of GAB1 and phosphorylated GAB1 are similar regardless of the level of expression of HSP70 (Fig. 3, middle panel).

Interestingly, the SHP2 bound to GAB1 were dephosphorylated in HSP70 transfected cell lysates even though the time dependent SHP2 dissociation from GAB1 was very slow after EGF treatment (Fig. 3, middle panel). In contrast the GAB1 bound to SHP2 was dephosphorylated and dissociated from SHP2 quickly in HSP70 transfected cells. Interestingly the binding of PI3K with GAB1 almost does not change in the HSP70 overexpression samples as compared to the non-transfected cells (Fig. 3, middle panel). Therefore SHP2 together with HSP70 appears to regulate the binding of SHP2 with GAB1 but has little influence on the binding of GAB1 with PI3K after EGF treatment.

This may indicate that HSP70 levels and interaction with SHP2 may have some influence on this EGF signaling pathway.

HSP70 Regulate SHP2 Binding with GAB1 and EGFR in vivo

To confirm HSP70 may affect binding of endogenous SHP2 with GAB1 and EGFR, a comparison of non-transfected or *HSP70* transfected cell lysates was performed using IP with anti-SHP antiserum with (without) EGF treatment (Fig 4B). Endogenous EGFR, GAB1 and SHP2 level is similar (Fig 4A). Under these conditions, we performed IP with anti-SHP2 antiserum (Fig 4B). SHP2 levels are similar in all cases, HSP70 protein binding with SHP2 depends on HSP70's expression. EGFR binding with endogenous SHP2 is decreased in HSP70 over-expressed cells (Fig 4B). Moreover, GAB1 binding with SHP2 increased on EGF addition but almost disappeared in HSP70 transfected cell lysates (Fig 4B). These data indicated that HSP70 levels regulate SHP2's interactions with these two endogenous substrates *in vivo*.

HSP70 binds with SHP2 in the Cell Membrane after EGF treatment

To confirm that the SHP2 and HSP70 proteins are binding in the cell and to try and localize the region within the cell where they could interact we tried co-immunostaining. The SHP2 transfected cells were co-immunostained with antibodies specific to the SHP2 protein and HSP70 with or without EGF treatment. Endogenous HSP70 is localized in the cytosol region as small spots and exogenous SHP2 localized in the cytosol with no specific localization before EGF treatment (Fig 4C, upper). Because of the diffuse cytoplasmic nature of these signals it is not possible to determine if these two proteins co-localize prior to EGF treatment. However, when cells are treated with EGF some HSP70 now clearly co-localized with SHP2 in the cell plasma membrane region (Fig 4C, lower). Secondary antibody only or mouse IgG only treated cells did not show any specific signal (Data not shown). These results indicate that a subpopulation of SHP2 and HSP70 molecules move into the cell membrane region on EGF treatment. Therefore although the binding of SHP2 and HSP70 appears to be constitutive and not affected by EGF treatment, it is possible that at least the intracellular location of a subpopulation of these molecules is affected by EGF treatment

DISCUSSION

The present study reports that SHP2, a protein tyrosine phosphatase that functions in the EGF signal pathway, interacts with HSP70 protein. It appears that HSP70 is a binding partner and not a substrate of SHP2. However, this binding may influence the EGF signaling pathway. The importance of SHP2 on signaling by several growth factors has been studied extensively [1, 2,5]. Diverse roles of SHP2 have been predicted from previous reports in which SHP2 binds several proteins [7,15-18]. We believe that the current study expands the complex biological regulation of SHP2 in EGF signaling pathways.

The biological significance of HSP70 interaction with SHP2 need to be studied more. However, our results indicated that HSP70 together with SHP2 regulates EGF signaling pathways. Here, we found that interaction of HSP70 with SHP2 regulates EGF signaling pathway through regulation of EGFR-SHP2 and/or GAB1-SHP2 interactions. In this report, SHP2 binds with HSP70 *in vivo*, and these proteins co-localized in the cell membrane after EGF treatment (Fig. 4C). The binding capacity of HSP70 with SHP2 is not changed after EGF treatment, but the binding capacity of EGFR with SHP2 is weaker under the same conditions. The SHP2-HSP70 interaction is not an enzyme/substrate interaction, neither does it involve tyrosine phosphorylation and thus is different from SHP2-EGFR and/or SHP2-GAB1 interactions (Fig. 2 and 3). Based on the immunofluorescence experiments it is possible that the SHP2/HSP70 complex relocate from the cytosol to the cell membrane after EGF treatment. This may indicate that EGF affects the localization of HSP70/SHP2 complex thus regulating some SHP2 related signal.

It is interesting to note the affect of HSP70 on SHP2 substrate interactions. Our present findings suggest that HSP70 has differential and relatively specific effects on SHP2 related signaling pathways. HSP70 expression in cells results in a temporal and differential inhibition of cell activation [19]. Our results imply that HSP70 could induce an increase in tyrosine phosphatase activity of SHP2 shifting the balance of phosphorylation and dephosphorylation, resulting in a decrease in tyrosine phosphorylation of essential binding molecules of SHP2. It seems that binding of SHP2DM with EGFR was not affected by HSP70 levels. This is consistent with a hypothesis that the effects of the HSP70/SHP2 interaction require the catalytic activity of SHP2 and not just protein-protein binding interactions.

Previously, SHP2 was found to down-regulate PI3K activation by dephosphorylation of GAB1 after EGF treatment [20]. However, the binding level of PI3K with GAB1 was not noticeably affected by increased levels of HSP70 (Fig. 3). This indicates that there is clear specificity in the effect of the HSP70/SHP2 complex on SHP2 substrate interactions.

The physiological meaning of SHP2's binding with HSP70 needs to be elucidated further. Nevertheless, this study demonstrates that HSP70 is a binding partner of SHP2, and that this binding can affect the binding of SHP2 with known SHP2's substrates, like EGFR and GAB1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by grant P01 CA-28146 from the National Institutes of Health to M.J.H.

REFERENCES

1. Agazie YM, Hayman MJ. Molecular mechanism for a role of SHP2 in epidermal growth factor receptor signaling. *Mol Cell Biol* 2003;23:7875–7886. [PubMed: 14560030]

2. Klinghoffer RA, Kazlauskas A. Identification of a putative Syp substrate, the PDGF beta receptor. *J Biol Chem* 1995;270:22208–22217. [PubMed: 7545675]
3. Ren Y, Meng S, Mei L, Zhao ZJ, Jove R, Wu J. Roles of Gab1 and SHP2 in paxillin tyrosine dephosphorylation and Src activation in response to epidermal growth factor. *J Biol Chem* 2004;279:8497–8505. [PubMed: 14665621]
4. Zhang SQ, Yang W, Kontaridis MI, Bivona TG, Wen G, Araki T, Luo J, Thompson JA, Schraven BL, Philips MR, Neel BG. Shp2 regulates SRC family kinase activity and Ras/Erk activation by controlling Csk recruitment. *Mol Cell* 2004;13:341–355. [PubMed: 14967142]
5. Hanafusa H, Torii S, Yasunaga T, Matsumoto K, Nishida E. Shp2, an SH2-containing protein-tyrosine phosphatase, positively regulates receptor tyrosine kinase signaling by dephosphorylating and inactivating the inhibitor Sprouty. *J Biol Chem* 2004;279:22992–22995. [PubMed: 15031289]
6. Wu CJ, O'Rourke DM, Feng GS, Johnson GR, Wang Q, Greene MI. The tyrosine phosphatase SHP-2 is required for mediating phosphatidylinositol 3-kinase/Akt activation by growth factors. *Oncogene* 2001;20:6018–6025. [PubMed: 11593409]
7. Zhang SQ, Tsiras WG, Araki T, Wen G, Minichiello L, Klein R, Neel BG. Receptor-specific regulation of phosphatidylinositol 3'-kinase activation by the protein tyrosine phosphatase Shp2. *Mol Cell Biol* 2002;22:4062–4072. [PubMed: 12024020]
8. Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev* 1998;12:3788–3796. [PubMed: 9869631]
9. Bukau B, Deuerling E, Pfund C, Craig EA. Getting newly synthesized proteins into shape. *Cell* 2000;101:119–122. [PubMed: 10786831]
10. Frydman J. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 2001;70:603–647. [PubMed: 11395418]
11. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 2002;295:1852–1858. [PubMed: 11884745]
12. Agazie YM, Hayman MJ. Development of an efficient “substrate-trapping” mutant of Src homology phosphotyrosine phosphatase 2 and identification of the epidermal growth factor receptor, Gab1, and three other proteins as target substrates. *J Biol Chem* 2003;278:13952–13958. [PubMed: 12582165]
13. Park CY, Hayman MJ. The tyrosines in the bidentate motif of the env-sea oncoprotein are essential for cell transformation and are binding sites for Grb2 and the tyrosine phosphatase SHP-2. *J Biol Chem* 1999;274:7583–7590. [PubMed: 10066827]
14. Yoo JC, Chang JR, Kim SH, Jang SK, Wolgemuth DJ, Kim K, Rhee K. NIP1/XB51/NECAB3 is a potential substrate of Nek2, suggesting specific roles of Nek2 in Golgi. *Exp Cell Res* 2004;292:393–402. [PubMed: 14697346]
15. Bardelli A, Longati P, Gramaglia D, Stella MC, Comoglio PM. Gab1 coupling to the HGF/Met receptor multifunctional docking site requires binding of Grb2 and correlates with the transforming potential. *Oncogene* 1997;15:3103–3111. [PubMed: 9444958]
16. Carlberg K, Rohrschneider LR. Characterization of a novel tyrosine phosphorylated 100-kDa protein that binds to SHP-2 and phosphatidylinositol 3'-kinase in myeloid cells. *J Biol Chem* 1997;272:15943–15950. [PubMed: 9188495]
17. Feng GS, Shen R, Heng HH, Tsui LC, Kazlauskas A, Pawson T. Receptor-binding, tyrosine phosphorylation and chromosome localization of the mouse SH2-containing phosphotyrosine phosphatase Syp. *Oncogene* 1994;9:1545–1550. [PubMed: 8183548]
18. Gale NW, Kaplan S, Lowenstein EJ, Schlessinger J, Bar-Sagi D. Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras. *Nature* 1993;363:88–92. [PubMed: 8386805]
19. Mortaz E, Redegeld FA, van der Heijden MW, Wong HR, Nijkamp FP, Engels F. Mast cell activation is differentially affected by heat shock. *Exp Hematol* 2005;33:944–952. [PubMed: 16038788]
20. Yart A, Laffargue M, Mayeux P, Chretien S, Peres C, Tonks N, Roche S, Payrastre B, Chap H, Raynal P. A critical role for phosphoinositide 3-kinase upstream of Gab1 and SHP2 in the activation of ras and mitogen-activated protein kinases by epidermal growth factor. *J Biol Chem* 2001;276:8856–8864. [PubMed: 11134009]

FIG. 1.

Direct interaction of SHP2 and HSP70 and effect of EGF treatment in cell.

The binding level of SHP2 with HSP70 or other tyrosine phosphorylated proteins was assessed by IP. Cellular lysates of each construct transfected cell were immunoprecipitated with the SHP2 antiserum. After IP, the indicated antibodies were used for western blot.

FIG. 2.

Regulation of HSP70 on binding of SHP2 with EGFR.

(A) Western blot analysis was carried out with lysates of cells transfected with *SHP2WT* or *SHP2DM* with (without) *HSP70* constructs. Indicated antibodies were used to detect specific proteins.

(B) The cell lysates were immunoprecipitated with SHP2 antiserum, followed by western blot analysis using EGFR, SHP2, HSP70 or 4G10 antibodies.

FIG. 3.

HSP70 regulates binding of SHP2 with GAB1.

SHP2 and GAB1 (FLAG) were immunoprecipitated from cells that were transfected with SHP2WT/FGAB1 or SHP2WT/FGAB1/HSP70. The indicated antibodies were used for western blot.

FIG. 4.

HSP70 regulates binding of SHP2 with EGF and GAB1 *in vivo* and Co-localization of the SHP2 with HSP70 after EGF treatment.

(A) The lysates were obtained from cells that had been transfected transiently with the HSP70 constructs or vector control. The cell lysates were western-blotted with the indicated antibodies.

(B) The binding level of SHP2 with EGFR, GAB1, HSP70 or other tyrosine phosphorylated protein was assessed by IP with SHP2 antiserum. After IP, the indicated antibodies were used for western blot.

(c) COS-1 cells were co-immunostained with antibodies against HSP70 (A and E) and SHP2 (B and F). The nucleus was stained with DAPI (C and G). Panel D and H is a merged image. Cells in Panel E-H were treated with EGF (100ng/ml) for 10 min.