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SHP-2 inhibits tyrosine phosphorylation of Cas-L and regulates cell migration

Koji Yo [a], Satoshi Iwata [a], Yutaka Hashizume [a], Shunsuke Kondo [a], Sayaka Nomura [a], Osamu Hosono [a], Hiroshi Kawasaki [a], Hirotoshi Tanaka [a], Nam H. Dang [b], Chikao Morimoto [a,*]

[a] Division of Clinical Immunology, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[b] Department of Hematologic Malignancies, Nevada Cancer Institute, One Breakthrough Way, Las Vegas, NV 89135, USA

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ABSTRACT

The Src homology 2 (SH2) domain-containing protein tyrosine phosphatase, SHP-2, plays an important role in cell migration by interacting with various proteins. In this report, we demonstrated that SHP-2 inhibits tyrosine phosphorylation of Crk-associated substrate lymphocyte type (Cas-L), a docking protein which mediates cell migration, and found that SHP-2 negatively regulates migration of A549 lung adenocarcinoma cells induced by fibronectin (FN). We showed that overexpressed SHP-2 co-localizes with Cas-L at focal adhesions and that exogenous expression of SHP-2 abrogates cell migration mediated by Cas-L. SHP-2 inhibits tyrosine phosphorylation of Cas-L, and associates with Cas-L to form a complex in a tyrosine phosphorylation-dependent manner. Finally, immunoprecipitation experiments with deletion mutants revealed that both SH2 domains of SHP-2 are necessary for this association. These results suggest that SHP-2 regulates tyrosine phosphorylation of Cas-L, hence opposing the effect of kinases, and SHP-2 is a negative regulator of cell migration mediated by Cas-L.

Introduction

Cas-L/neural precursor cell expressed, developmentally down-regulated 9 (NEDD9)/human enhancer of filamentation 1 (HEF1) (hereafter designated Cas-L) is a multifunctional scaffolding protein that belongs to the Cas family [1]. It has diverse functions, including having a role in the regulation of cell migration [2–6], invasion [3,7], cell cycle [8–11], and apoptosis [12,13]. With regard to cell migration, we and others have reported that Cas-L is tyrosine phosphorylated on stimulation with integrin and growth factor receptors, and tyrosine phosphorylated Cas-L interacts with Crk, Nck, and C3G [1,14]. This interaction is supposed to result in the activation of small GTPases, which promotes cell migration. Furthermore, recent publications have reported novel findings on the role of Cas-L in tumor progression [3,7]. These reports hence demonstrate the crucial role of Cas-L in the pathogenesis of tumor metastasis *in vivo*.

With regard to the regulation of Cas-L tyrosine phosphorylation, while the role of kinases such as FAK and Src has been well-understood, little is known about phosphatases which regulate tyrosine phosphorylation in an opposite manner. In a previous study, Minegishi et al. reported that SHP-2 associates with Cas-L, and this finding leads us to our present effort to investigate the interaction between SHP-2 and Cas-L.

SHP-2 is a ubiquitously expressed cytoplasmic, protein tyrosine phosphatase (PTP) that has two SH2 domains at the N-terminal region, a PTP domain, and a COOH-terminal tail. There is much evidence that SHP-2 transduces signals from growth factors and cytokines, and regulates the cell migration. For example, Ren et al. reported that SHP-2 associates with Gab1, and is involved in epidermal growth factor (EGF)-induced paxillin dephosphorylation and Src tyrosine kinase activation [15]. Manes et al. reported that SHP-2 dephosphorylates FAK and increases cell migration by promoting turnover of focal adhesions in insulin-like growth factor (IGF)-1-stimulated MCF-7 cells [16]. In these reports, SHP-2 thus functions as a positive regulator of cellular migration. On the other hand, other investigators have reported that SHP-2 can also function as a negative regulator of cell migration [17–19]. Overall, the role of SHP-2 in cell migration appears to be dependent on the assay system.

In this study, we investigate the mechanisms involved in SHP-2 regulation of Cas-L-mediated cell migration and its tyrosine phosphorylation, with the goal of obtaining novel findings relating to SHP-2 function.

Experimental procedures

Reagents and antibodies. Mouse monoclonal antibody (mAb) against Cas-L was from ImmuQuest (Cleveland, UK). Anti-SHP-2

Abbreviations: SHP-2, Src homology 2 domain-containing protein tyrosine phosphatase 2; Cas-L, Crk-associated substrate lymphocyte type.

* Corresponding author. Fax: +81 354 495 448.
E-mail address: morimoto@ims.u-tokyo.ac.jp (C. Morimoto).

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mAb was purchased from BD Biosciences (Franklin Lakes, NJ, USA). Anti-myc mAb (9E10) and anti-phosphotyrosine (pTyr) mAb (4G10) were produced from hybridoma obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Anti-Flag mAb was purchased from Sigma–Aldrich (St. Louis, MO, USA). All the chemicals and reagents were obtained from Sigma–Aldrich unless otherwise stated.

Cells, plasmids, and transfection procedures. 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) at 37 °C with 5% CO_2. A549 cells were cultured in RPMI 1640 containing 10% FCS at 37 °C with 5% CO_2. c-myc-tagged Cas-L in pEB6 vector was described previously [20]. SHP-2 cDNA was purchased from OriGene Technologies, Inc. (Rockville, MD, USA). pEB6-Flag-SHP-2 mutants, in which the cysteine 459 was changed to serine and/or aspartic acid 425 to alanine using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA), which were designated as SHP-2 CS (catalytically inactive mutant [21]), SHP-2 DA and SHP-2 DM (DA/CS) (substrate-trapping mutant [22]), respectively. CAGGS-Flag SHP-2 CS ΔN-SH2 lacking aa 1–107, ΔN/C-SH2 lacking aa 1–216, ΔPTP lacking aa 214–597 were generated by PCR, using the primers containing the restriction sites for EcoRI and XhoI (ΔN-SH2: forward primer 5′-CGGAATTCACCTCTGAAAGGTGGT-3′, reverse primer 5′-CCCTCG AGTTGCGTCTGTTCTTG-3′; ΔN/C-SH2: forward primer 5′-CGGA ATTCAACACGACTCGTATAAATGCTGCTGAAATAG-3′, reverse primer 5′-CCCTCGAGCATAAACTTTCTTGCGTCTGTTCTTGATCTT-3′; ΔPTP: forward primer 5′-CGGAATTCACATCGCGGAGATGGT-3′, reverse primer 5′-CCCTCGAGTCAGTCAGTCATGTTAAGGGGCTGCTT-3′).

To generate constitutively active Fyn, tyrosine 528 was substituted by phenylalanine by introducing a punctual mutation into the 3′ primer. The resultant mutant Fyn (Y528F) was designated as Fyn (CA). The same method was employed to generate the following mutants: Fyn (K299M) as Fyn (KN) (KN, kinase negative). The plasmids were transfected into cells using Lipofectamine LTX with PLUS reagent (Invitrogen) according to the manufacturer's instructions.

Immunocytochemistry. For fluorescent microscopy experiments using A549 cells, the cells were grown on coverslips, and transfected with c-myc-tagged Cas-L alone, or together with Flag-tagged SHP-2, and were treated and stained as described previously [6].

Migration assay. A549 cell migration was assayed using 6.5 mm-diameter Transwell inserts (Corning, Inc., Life Science, Acton, MA, USA) with FN-coated polycarbonate filters (8.0 μm pore size) as previously described [4].

Immunoprecipitation and immunoblotting. The cells were lysed and subjected to immunoprecipitation and immunoblotting as previously described [6].

In vitro Cas-L tyrosyl dephosphorylation. GST (glutathione *S*-transferase)-SHP-2 fusion proteins used in the Cas-L dephosphorylation assay were kind gifts from Dr. Anton M. Bennett (Yale University School of Medicine), and purified using Microspin GST Purification Module (Amersham Biosciences), according to the manufacturer's instructions. Tyrosine-phosphorylated Cas-L was immunoprecipitated from A549 cellular lysates using anti-Cas-L antibodies, incubated with GST fusion proteins as previously described [23], subjected to immunoblotting.

Results

Exogenous SHP-2 co-localizes with Cas-L at focal adhesions

We first examined subcellular localization of SHP-2 in A549 human lung carcinoma cells. As previously reported, Cas-L co-localized with paxillin at focal adhesions [24,13] (Fig. 1, upper). Co-transfection studies of Flag-tagged SHP-2 with c-myc-tagged Cas-L analyzed by confocal microscopy showed that SHP-2 and Cas-L co-localized at focal adhesions (Fig. 1, lower). These observations suggested that SHP-2 may associate with Cas-L at focal adhesions and have a role in integrin-dependent functions of Cas-L.

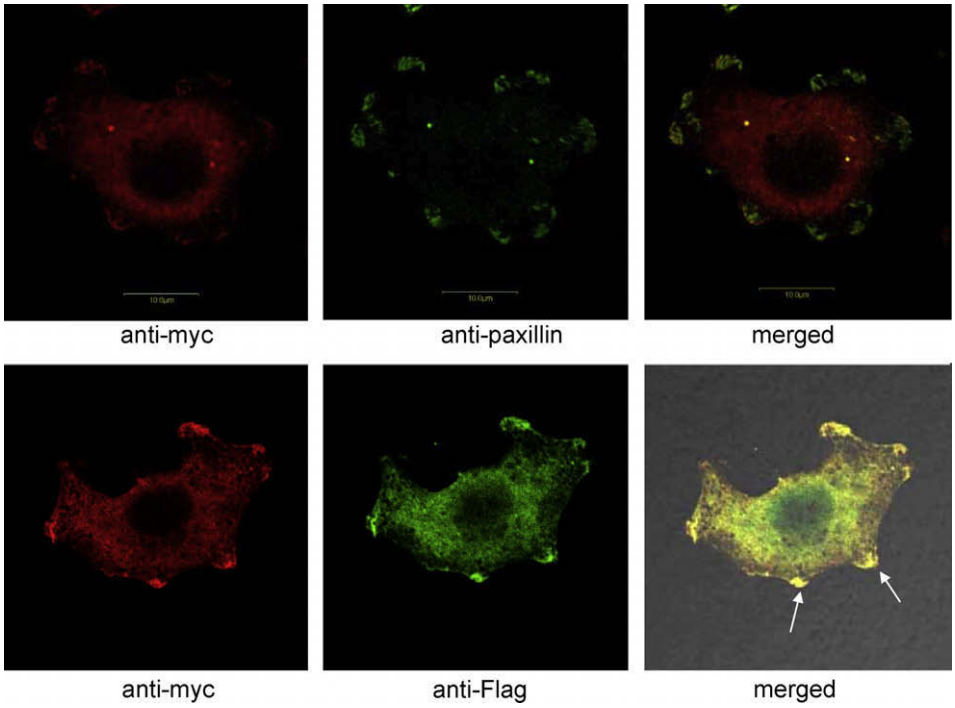

Fig. 1. Overexpressed SHP-2 co-localizes with Cas-L at focal adhesions. (Upper panel) A549 cells were transfected with plasmids expressing c-myc-tagged Cas-L, followed by immunocytochemistry using the indicated antibodies. Paxillin is indicated in green, and Cas-L in red. (Lower panel) A549 cells were transfected with plasmids expressing c-myc-tagged Cas-L and Flag-tagged SHP-2. SHP-2 is indicated in green, and Cas-L in red. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)