Insulin receptor substrate protein 53 (IRSp53) as a binding partner of antimetastasis molecule NESH, a member of Abelson interactor protein family

Involvement of NESH in cancer invasion and metastasis is now very important and interesting. In order to clarify the molecular mechanisms, we identified insulin receptor substrate protein 53 (IRSp53) as a partner of NESH utilizing a conventional yeast two-hybrid screen with a proline-rich domain of NESH as bait. The specific interaction was confirmed by coimmunoprecipitation assay and colocalization analysis in mammalian cells. The carboxyl-terminal SH3 domain of IRSp53 is responsible for the interaction with the proline-rich domain of NESH. NESH may play a critical role in regulating the actin cytoskeleton required for membrane ruffling induced by the IRSp53 pathway.

We observed that NESH expression caused a marked reduction in motility and exhibited significant reduction in tumor metastatic potential in vivo [1]. NESH expression is also frequently lost in invasive cancer cell lines despite its ubiquitous expression in normal tissues. We, however, also found that use of imatinib to treat NESH-expressed cancer cells greatly enhances its invasive tumor growth in vivo [2]. An unknown antimetastasis pathway in which NESH is involved might be regulated by abelson tyrosine kinase [3]. To identify proteins interacting with the NESH, we used a two-hybrid system to screen a human complementary DNA library with a NESH protein as bait. More than four positive preys were identified, and subsequent sequence analysis revealed one of the preys to be IRSp53.

Figure 1. Interaction between the full-length IRSp53 and NESH. (A) Coimmunoprecipitation of IRSp53 with NESH. NIH3T3 cell lysates were immunoprecipitated by anti-NESH (upper panel) or anti-IRSp53 (lower panel) antibodies, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot analysis with anti-IRSp53 or anti-NESH antibodies, respectively. (B) Colocalization of NESH and IRSp53. The 3Y1 cells were transiently transfected with pcDNA3.1-NESH. The cells were then fixed and incubated with anti-NESH and anti-IRSp53 antibodies followed by staining with fluorescein isothiocyanate (FITC)-conjugated and rhodamine-conjugated secondary antibodies. Images were obtained using a confocal microscope. The yellow color in the merged image represents the extent of the localization of NESH (green) and IRSp53 (red). (C) The NIH3T3 cells were transiently transfected with pcDNA3.1-NESH. After 2 days, the serum-starved cells were stimulated with or without 5 ng/ml PDGFbb for 10 min. The cells were then fixed and incubated with anti-NESH antibody followed by staining with FITC-conjugated secondary antibody and rhodamine phalloidin to visualize actin filaments. The merged figures show images of green (green fluorescent protein-labeled proteins) and red rhodamine labeling obtained for the same section. White arrows indicate the membrane ruffling. (D) The membrane ruffling ratio with (black bar) or without (hatched) NESH expression cells. Data are the means ± SDs of 200 independent cells. *P < 0.001.
To determine whether IRSp53 and NESH might interact, we first determined the association by glutathione S-transferase (GST) pull-down assays. We found that the SH3 domain of IRSp53 was associated with the proline-rich region of NESH in NIH3T3 cells but not with control GST protein (data not shown). We examined the biochemical in vivo interactions between NESH and IRSp53 (Figure 1A). NIH3T3 cells were transfected with expression plasmids for NESH. The cell lysates were subjected to immunoprecipitation using either anti-NESH or anti-IRSp53 antibodies. As shown in Figure 1A, NESH, but not control, was coprecipitated with IRSp53. Reciprocally, IRSp53 was coprecipitated with NESH but not with the mock antibody. We next carried out double immunofluorescent staining for IRSp53 and NESH in 3Y1 cells. Analysis with confocal microscopy clearly indicated that NESH and IRSp53 colocalized in 3Y1 cells (Figure 1B). We also investigated the subcellular localization of IRSp53 in NIH3T3 and human umbilical vein endothelial cells, and the IRSp53 staining again overlapped with that of NESH (data not shown). These results indicated an interaction between IRSp53 and NESH in vivo. The two-hybrid assay revealed that the SH3 region of IRSp53 was necessary for this interaction since the truncated protein of IRSp53 did not interact with NESH (data not shown). Several studies have indicated the involvement of IRSp53 in growth factor-mediated membrane ruffling. Therefore, we examined the effects of NESH expression on platelet-derived growth factor (PDGF)-induced membrane ruffling. Treating the cells with 5 ng/ml of PDGF for 10 min induced rapid membrane ruffling that was not present in untreated cells (Figure 1C, 1–2). However, when the cells were transiently transfected with the NESH expression plasmid, PDGF-mediated membrane ruffling was undetectable (Figure 1C, 3–5, and D).

Tumor metastasis is a dynamic multistep process involving increased migration to the distant site. A variety of molecules function and interact with each other in order to metastasize. NESH as well as IRSp53 is localized to the edge of the lamellipodial protrusions, in which the migration requires polymerization of actin. IRSp53 can associate with Rac as small guanosine triphosphatases of the Rho family through its amino-terminal domain and plays a pivotal role in lamellipodia formation in motile cells [4]. Binding of activated Rac and WAVE2 via IRSp53 induces actin filament assembly, reorganization and membrane ruffling in NIH3T3 cells. The manner of regulation of this activity, however, remains largely unknown. Here, we show that overexpression of NESH potently blocks PDGF-stimulated membrane ruffling in mammalian cells. NESH binds IRSp53-SH3 domain which is identical with binding site of WAVE2 [5], which might compete with WAVE2 for binding to IRSp53.

funding

Grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology in Japan and Nara Women’s University Intramural Grant for Project Research.

S. Matsuda, S. Yokozaki, H. Yoshida, Y. Kitagishi, N. Shirafuji & N. Okumura

1Department of Environmental Health, Nara Women’s University, Nara 630-8506, 2Department of Molecular Pathogenesis, 3Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466-8550, 4Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan

†These authors contributed equally to this work.

(*E-mail: smatsuda@cc.nara-wu.ac.jp)

acknowledgements

The authors declare that they have no competing financial interests.

references


doi:10.1093/annonc/mdn293

Published online 13 May 2008