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Supplemental materials and methods

cDNAs and their expression

pEF-BOS-HAx3 Rac1-G12V and -T17N were gifts from Dr. K. Kaibuchi (Nagoya University, Nagoya, Japan). HA-tagged mouse Fak mutant cDNAs, K454R, Y397F, Y407F, and Y861F have been described previously (Polte and Hanks 1997). Mouse Fak ΔFAT cDNA (deletion of aa 929–1052) was constructed using the PCR amplification method. EGFP-paxillin cDNA was described previously (Mazaki et al., 1998), and the rescue cDNA was constructed by substituting the nucleotides within the siRNA target to 5′-CTCTTACTAAGGAAAAACCCT-3′. pRaichu-Rac was described previously (Itoh et al., 2002). pCX4-FRNK-HA was a gift from Dr. T. Iwahara (Osaka Bioscience Institute, Osaka, Japan). cDNA transfection was done using PolyFect® (QIAGEN) according to the manufacturer's instructions. For cotransfection of siRNAs and cDNAs, cells were transfected with cDNAs 24 h after the transfection with siRNAs, and were incubated for a further 24 h before being subjected to analyses. Exogenous Fak and paxillin proteins were detected by staining the HA tag and by detecting the autofluorescence from the EGFP tag, respectively.

Antibodies and others

The anti-paxillin antibody was described previously (Mazaki et al., 1998). Antibodies against the following proteins were purchased from commercial sources: E-cadherin (TaKaRa); N-cadherin, p130Cas, paxillin, Pyk2, and α-, β-, γ-, and p120-catenin (TDL); Fak clone 2A7 and 4.47 and phosphotyrosine clone 4G10 (UBI); β1-integrin clone MAB 2247 (CHEMICON International); Pan-cadherin and vinculin (Sigma-Aldrich); HA-tag clone 16B12 (BAbCO); phosphorylation site-directed anti-Fak antibodies (QCB); affinity-purified donkey antibodies to rabbit or mouse IgG conjugated either with HRP, Cy2, Cy3, or Cy5 (Jackson ImmunoResearch Laboratories); and Alexa® 350–conjugated goat antibody to mouse IgG (Molecular Probes, Inc). The DGEA and RGDS peptides were from Bachem and Sigma-Aldrich, respectively. The Cy3-based siRNA labeling kit was from Ambion.

Immunoblotting

Immunoblotting was performed as described previously (Tsubouchi et al., 2002). Amounts of proteins in siRNA-treated cells were measured by a densitometer (GT8700F scanner; Epson) using NIH Image version 1.63 software (National Institutes of Health, Bethesda, MD).

Phase-contrast video images

A time-lapse video recording was done at an interval of 1 min, using a microscope (AxioVert 200; Carl Zeiss MicroImaging, Inc.) equipped with a camera system (AxioCam; Carl Zeiss MicroImaging, Inc.), a shutter unit (Uniblitz), and a CO_2 incubator unit (CZI-3; Carl Zeiss MicroImaging, Inc.). Phase-contrast video images, visualized using the computer software attached to the AxioCam system (ImageBrowser version 5.1), were subjected to counting of aberrant large protrusions or tracing of cell migration.

References

Andrieux, A., G. Hudry-Clergeon, J.J. Ryckewaert, A. Chapel, M.H. Ginsberg, E.F. Plow, and G. Marguerie. 1989. Amino acid sequences in fibrinogen mediating its interaction with its platelet receptor, GPIIbIIIa. *J. Biol. Chem.* 264:9258–9265.

Itoh, R.E., K. Kurokawa, Y. Ohba, H. Yoshizaki, N. Mochizuki, and M. Matsuda. 2002. Activation of Rac and Cdc42 video imaged by fluorescent resonance energy transfer-based single-molecule probes in the membrane of living cells. *Mol. Cell. Biol.* 22:6582–6591.

Mazaki, Y., H. Uchida, O. Hino, S. Hashimoto, and H. Sabe. 1998. Paxillin isoforms in mouse: lack of the γ isoform and developmentally specific β isoform expression. *J. Biol. Chem.* 273:22435–22441.

Polte, T.R., and S.K. Hanks. 1997. Complexes of focal adhesion kinase (FAK) and Crk-associated substrate (p130Cas) are elevated in cytoskeleton-associated fractions following adhesion and Src transformation: requirements for Src kinase activity and Fak proline-rich motifs. *J. Biol. Chem.* 272:5501–5509.

Staatz, W.D., K.F. Fokg, M.M. Zutter, S.P. Adamss, B.A. Rodriguez, and S.A. Santoro. 1991. Identification of a tetrapeptide recognition sequence for the $\alpha_2\beta_1$ integrin in collagen. *J. Biol. Chem.* 266:7363–7367.

Tsubouchi, A., J. Sakakura, R. Yagi, Y. Mazaki, E. Schaefer, H. Yano, and H. Sabe. 2002. Localized suppression of RhoA activity by Tyr31/118-phosphorylated paxillin in cell adhesion and migration. *J. Cell Biol.* 159:673–683.