p130<sup>CAS</sup> Forms a Signaling Complex with the Adapter Protein CRKL in Hematopoietic Cells Transformed by the BCR/ABL Oncogene*

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The Philadelphia chromosome (Ph) translocation generates a chimeric tyrosine kinase oncogene, BCR/ABL, which causes chronic myelogenous leukemia (CML) and a type of acute lymphoblastic leukemia (ALL). In primary samples from virtually all patients with CML or Ph<sup>+</sup> ALL, the CRKL adapter protein is tyrosine phosphorylated and physically associated with p130<sup>CAS</sup> (Crk associated substrate). CRKL has one SH2 domain and two SH3 domains and is structurally related to c-CRK-II (CRK) and the v-Crk oncogene. We have previously shown that CRKL binds to BCR/ABL, that CRKL binds to BCR/ABL through the CRKL-SH3 domain in p130<sup>CAS</sup> containing Tyr-terminus of the molecule (16). p130<sup>CAS</sup> also has been shown to bind one or more cellular proteins, one of which is p120<sup>CBL</sup>. Here we demonstrate that another cellular protein linked to BCR/ABL through the CRKL-SH2 domain is p130<sup>CAS</sup>. p130<sup>CAS</sup> was found to be tyrosine phosphorylated and associated with CRKL in BCR/ABL expressing cell lines and in samples obtained from CML and ALL patients, but not in samples from controls. In both normal and BCR/ABL transformed cells, p130<sup>CAS</sup> was detected in focal adhesion-like structures, as was BCR/ABL. In normal cells, the focal adhesion proteins tensin, p125<sup>FAK</sup>, and paxillin constitutively associated with p130<sup>CAS</sup>. However, in BCR/ABL transformed cells, the interaction between p130<sup>CAS</sup> and tensin was disrupted, while the associations between p130<sup>CAS</sup>, p125<sup>FAK</sup>, and paxillin were unaffected. These results suggest that the BCR/ABL oncogene could alter the function of p130<sup>CAS</sup> in at least three ways: tyrosine phosphorylation, inducing constitutive binding of CRKL to a domain in p130<sup>CAS</sup> containing Tyr-X-Pro motifs (substrate domain), and disrupting the normal interaction of p130<sup>CAS</sup> with the focal adhesion protein tensin. These alterations in the structure of signaling proteins in focal adhesion like structures could contribute to the known adhesion abnormalities in CML cells.

Chronic myelogenous leukemia (CML) and some acute lymphoblastic leukemias (ALL, Ph<sup>+</sup>) are caused by the activated tyrosine kinase fusion protein BCR/ABL. There are multiple proteins which are tyrosine phosphorylated in response to BCR/ABL transformation (1–4). We and others have shown that one of the most prominent and consistently tyrosine-phosphorylated proteins is an adapter protein termed CRKL (CRK-like) (5–7). CRKL has a 60% homology to c-CRK-II (CRK), the human homologue of v-Crk (8, 9). The v-crk oncogene was originally cloned from the CT1 virus and can transform fibroblasts and other cells. The v-Crk oncoprotein contains both one SH2 and one SH3 domain, while c-Crk-II and CRKL contain one SH2 and two SH3 domains (10, 11). A second product of the c-crk gene, c-Crk-I, has one SH2 and one SH3 domain, but to date, only one form of CRKL has been observed and no oncogenic forms have been described. v-crk transformation in 3Y1 fibroblasts leads to tyrosine phosphorylation of multiple proteins (12). In the v-crk transformed fibroblasts, c-Abl has been shown to bind to v-Crk and is believed to be activated by this interaction (13).

Another prominent binding protein for the v-Crk oncoprotein has been shown to be p130<sup>CAS</sup> (Crk associated substrate) (14). p130<sup>CAS</sup> was originally cloned from the v-crk transformed 3Y1 cell line and shown to be heavily tyrosine phosphorylated in these cells (14, 15). Analysis of cDNA for p130<sup>CAS</sup> reveals multiple domains. The N terminus contains an SH3 domain, followed by a "substrate domain" consisting of 14 Tyr-X-X-Pro (Y-X-X-P) motifs, each of which are potential CRK/CRKL-SH2 binding sites (15). There is also a pp60<sup>Src</sup>-Src binding site in the C terminus of the molecule (16). p130<sup>CAS</sup> also has been shown to be tyrosine phosphorylated in response to v-Src (14, 17, 18). In v-src transformed cells, p130<sup>CAS</sup> is localized to focal adhesions (18, 19), and recently, several laboratories have shown that p130<sup>CAS</sup> is tyrosine phosphorylated in response to β1 integrin cross-linking (17, 18, 20, 21). Although the function of p130<sup>CAS</sup> in either normal or transformed cells is unknown, these observations suggest that p130<sup>CAS</sup> may be involved in integrin signaling.

Since p130<sup>CAS</sup> apparently plays a prominent role in transformation by the v-crk oncogene, we examined the interaction of p130<sup>CAS</sup> with c-Crk and CRKL in hematopoietic cells transformed by BCR/ABL and in normal hematopoietic cells. We find that p130<sup>CAS</sup> is tyrosine phosphorylated in most cell lines transformed by BCR/ABL and associated with the SH2 domain of CRKL, but not with c-Crk. This potentially links p130<sup>CAS</sup> to BCR/ABL since CRKL is constitutively associated with BCR/ABL through its SH3 domain. This interaction was unique to BCR/ABL transformed cell lines and was not observed in normal cells. Furthermore, in primary cells from patients with CML and Ph<sup>+</sup> acute lymphocytic leukemia (ALL), p130<sup>CAS</sup> co-immunoprecipitates with CRKL. In both...
normal and BCR/ABL transfected cells, we found that p130CAS localized in focal adhesion-like structures, as did BCR/ABL. Our results are of interest as they extend the known interactions between BCR/ABL and cellular signal transduction molecules mediated by CRKL.

MATERIALS AND METHODS

Cells, Cell Lines, and Cell Culture—The murine myeloid cell line 32Dcl3, murine pre-B cell line BaF3, human megakaryocytic cell line Mo7e, human T-cell line H9, human B-cell line Namal, and CML cell lines BV-173, and K562 were maintained in culture as described previously (22). The BCR/ABL expressing hematopoietic cell lines were generated by transfection with p210BCR/ABL or p190BCR/ABL DNA and maintained as described previously (22). NIH-3T3 cells and NIH-3T3 expressing p210BCR/ABL (courtesy of Dr. Warren Pear, MIT, Cambridge, MA) were maintained with 10% fetal calf serum in RPMI 1640. Normal neutrophils, thymocytes, mononuclear cells, and cells from CML, ALL, and chronic myelomonocytic leukemia patients were obtained with informed consent and approved IRB protocols, and isolated as described (22). A spleen sample was also obtained from a patient with CML and cells isolated by density gradient sedimentation.

Immunoblotting and Immunoprecipitations—Protein lysates were prepared as described previously, and used for immunoblotting or immunoprecipitations (24). Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred and immunoblotted as described using a chemiluminescence technique (25). Immunoprecipitation detection of tyrosine-phosphorylated proteins in Western blotting utilized monoclonal antibody 4G10 (a generous gift from Dr. Brian Druker, Oregon Health Science University, Portland, OR). Polyclonal rabbit antisera against tensin (R95), p120CBL, in these cells and results are shown in Fig. 1. It was noted that an anti-p130CAS rabbit polyclonal antibody from Santa Cruz detects a single band at 130 kDa by ECL; whereas p130CAS associates with CRKL but not c-CRK in BCR/ABL transfected cells—Immunoblotting was used to investigate the expression and tyrosine phosphorylation of p130CAS in hematopoietic cells. p130CAS was detected in various hematopoietic cells (Fig. 1). The expression was compared to another protein, p120CBL, in these cells and results are shown in Fig. 1. It was noted that an anti-p130CAS rabbit polyclonal antibody from Santa Cruz detects a single band at 130 kDa by ECL; whereas an anti-p130CAS mouse monoclonal antibody from Transduction Laboratories detects both a major band at 130 kDa and a minor band at 105 kDa. The minor 105-kDa band could be a related CAS form or a cross-reacting protein such as Hef1 (28). In these experiments we have focused on the 130-kDa band identified by both antibodies as p130CAS.

To determine if p130CAS is tyrosine phosphorylated in BCR/ABL transformed cells, cell lysates were immunoprecipitated with anti-p130CAS antibody and membranes immunoblotted with anti-phosphotyrosine antibody. p130CAS was tyrosine phosphorylated in 32Dcl3 and BaF3 cells transformed by interleukin-3 (BaF3(–)), BaF3 stimulated cells with interleukin-3 (BaF3(+)1), BaF3 cells transformed by p190BCR/ABL (BaF3.p190), BaF3 cells transformed by p210BCR/ABL (BaF3.p210), BV-173 cells, K562 cells, unstimulated Mo7e cells (Mo7e (–)), NIH3T3 cells, and NIH3T3 cells transformed by p210BCR/ABL (NIH3T3.p210).
Obtained from normal, CML, and ALL (Ph expressing BCR/ABL, we examined cell lysates from samples with anti-p130CAS antibody (4G10). The cells used are unstimulated control, we performed p130CAS immunoprecipitations in cell lysates obtained from a patient with chronic myelomonocytic leukemia (a Ph negative chronic leukemia) and there was no apparent co-precipitation of p130CAS and CRKL. The reverse experiment, anti-CRK immunoprecipitation and p130CAS immunoblot, showed p130CAS coimmunoprecipitated with CRKL in BCR/ABL positive samples and not normal neutrophils. There was no difference in communoprecipitation of CRKL with p130CAS seen with the addition of growth factor granulocyte-macrophage colony-stimulating factor to the neutrophils. The SH2 domain of CRKL Can Associate with the Substrate Domain of p130CAS—To determine which region(s) of CRKL can associate with p130CAS, we performed precipitations with full-length CRKL-GST, CRKL-SH2-GST, CRKL-SH2-SH3(N)-GST, and CRKL-SH3(N)-SH3(C)-GST fusion proteins of lysates from BCR/ABL transformed cells. The SH3 domain(s) could associate with c-ABL (27), and the SH2 domain could precipitate p130CAS (Fig. 5). This was observed in 32Dc13 and BaF3 cells transformed with BCR/ABL. Since the substrate domain of p130CAS contains multiple, clustered, Tyr-X-X-Pro motifs, we sought to determine if the SH2 domain of CRKL associates with p130CAS at these sites. It is shown that the phosphorylated substrate domain precipitated with CRKL in lysates from 32D.p210BCR/ABL cells. As a control, the SH3 domain and unphosphorylated substrate domain did not precipitate with CRKL. To show direct binding of CRKL-SH2 domain to p130CAS, we also performed far Western blotting. As seen in Fig. 6, CRKL-SH2 but not CRKL-SH3 directly bound to p130CAS in BCR/ABL expressing BaF3 cells. This was also true for 32D.p210BCR/ABL cells (data not shown).

p130CAS Associates with Focal Adhesion Proteins in Normal Myeloid Cells, but These Complexes Are Disrupted by BCR/ABL Transformation—We have previously shown that BCR/ABL is localized in focal adhesion-like structures in myeloid cells (4). p130CAS cellular staining in myeloid cells expressing BCR/ABL also showed that p130CAS is localized in focal adhesion-like structures (data not shown and Ref. 4). Since BCR/ABL is known to alter focal adhesion structure, we examined the possible interaction of p130CAS with normal focal adhesion proteins. Immunoprecipitations with anti-tensin, anti-p125FAK, anti-vinculin, and anti-panaxillin were performed and then immunoblotted with anti-p130CAS antibody. p130CAS immunoprecipitated with tensin, p125FAK, and paxillin in normal cells; whereas, only p125FAK and paxillin immunoprecipitated with p130CAS in BCR/ABL expressing 32Dc13 and BaF3 cells (Fig. 7). Vinculin, talin, and α-actinin did not coprecipitate with p130CAS (Fig. 7, and data not shown). The reverse experiment, with p130CAS immunoprecipitations and various focal adhesion mutant did not coprecipitate with p130CAS (Fig. 7, and data not shown). The reverse experiment, with p130CAS immunoprecipitations and various focal adhesion
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FIG. 4. p130Cas can associate with CRKL in BCR/ABL containing cells from patients with CML or Ph⁺ ALL. A, immunoprecipitates of lysates from cells from normal or leukemia patients (20 × 10⁶ cells) with anti-CRKL antibody were processed as described under “Materials and Methods” and applied to a gradient SDS-PAGE gel (6–12%) and transferred to Immobilon-P membrane. The membrane was immunoblotted with anti-p130Cas antibody, and thereafter stripped and reprobed with anti-CRKL antibody. Lanes 1 and 2 are normal neutrophils unstimulated (-) and stimulated (+) with granulocyte-macrophage colony-stimulating factor GM-CSF, respectively. Lanes 3–8 are neutrophil samples from three separate patients with stable phase CML, unstimulated and stimulated alternately. B, immunoprecipitates of lysates from cells from normal or leukemia patients (20 × 10⁶ cells) with anti-p130Cas antibody were processed as described under “Materials and Methods” and applied to a gradient SDS-PAGE gel (6–12%) and transferred to Immobilon-P membrane. The membrane was immunoblotted with anti-CRKL antibody. Lanes 1 and 2 are neutrophils from two separate normal subjects, lanes 3 and 4 are blast samples from two separate ALL (Ph⁺) patients, lanes 5 and 6 are neutrophils from two separate CML patients in stable phase, lane 7 is a sample from a spleen from a patient with CML, and lane 8 is a sample from a patient with chronic myelomonocytic leukemia (CMML).

FIG. 5. Identification of CRKL binding domains to p130Cas, and identification of p130Cas binding domain to CRKL. A, precipitations with various GST fusion proteins of full-length CRKL and its domains (SH2, SH3-SH3, SH2-SH3) with 32D.p210BCR/ABL.26 cell lysates were processed as described under “Materials and Methods.” The membrane was immunoblotted with anti-ABL antibody (top panel), thereafter, stripped and probed with anti-p130Cas antibody (lower panel). Shown are precipitations with GST protein alone, GST-CRKL (full-length), GST-CRKL-SH2, GST-CRKL-SH3(N)-SH3(C), and GST-CRKL-SH3(N)-SH3(N); and whole cell lysate (W.C.L.). Molecular weights shown in kDa. B, precipitations with various GST fusion proteins of p130Cas and its domains (SH3 and substrate domain) with 32D.p210BCR/ABL.26 cell lysates were processed as described under “Materials and Methods.” The membrane was immunoblotted with anti-CRKL antibody. Shown are precipitations with GST alone, GST-p130Cas-SH3, GST-p130Cas-SH3 (substrate domain), GST-p130Cas-SH3 (substrate domain, phosphorylated). Molecular weights are shown in kDa.

FIG. 6. CRKL-SH2 and ABL-SH2 but not CRKL-SH3 can bind directly to p130Cas as determined by far Western blotting. Whole cell lysates (W.C.L.) of BaF3-p210BCR/ABL cells or immunoprecipitates of cell lysates of BaF3 or BaF3.p210BCR/ABL with anti-p130Cas antibody (I.P., 20 × 10⁶, last two lanes) were processed as described under “Materials and Methods” and applied to a 7.5% SDS-PAGE and transferred to Immobilon-P membranes. The membranes were processed for a far Western as described under “Materials and Methods” with the various GST fusion proteins: GST, ABL-SH2, CRKL-SH2, and CRKL-SH3.

DISCUSSION

Chronic myelogenous leukemia was the first disorder identified with a specific abnormal chromosomal translocation, the Philadelphia chromosome, creating a fusion of two genes, BCR and ABL (exons b2a2 or b3a2), and thus generating the oncoprotein p210BCR/ABL (30). In an alternative fusion of BCR and ABL (exons b1a2), there is production of the oncoprotein p190BCR/ABL which can be seen in some acute lymphoblastic leukemias (30). BCR/ABL is a potent tyrosine kinase which directly top130Cas as determined by far Western blotting. Whole cell lysates (W.C.L.) of BaF3-p210BCR/ABL cells or immunoprecipitates of cell lysates of BaF3 or BaF3.p210BCR/ABL with anti-p130Cas antibody (I.P., 20 × 10⁶, last two lanes) were processed as described under “Materials and Methods” and applied to a 7.5% SDS-PAGE and transferred to Immobilon-P membranes. The membranes were processed for a far Western as described under “Materials and Methods” with the various GST fusion proteins: GST, ABL-SH2, CRKL-SH2, and CRKL-SH3.

CRKL is a 39-kDa protein with one SH2 and two SH3 domains (9). It has a homology to c-CRK-II, and belongs to the CRK family of adapter proteins (37). The CRK family consists of v-Crk, c-CRK-II, c-CRK-I, and CRKL. CRKL and c-CRK-II contain an NH₂-terminal SH2 domain and two tandem SH3 domains at the COOH terminus. c-CRK-I and v-Crk both have a deletion of the most COOH-terminal SH3 (38). CRKL has been shown to have similar in vitro binding charac-
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FIG. 7. p130\(^{\text{CAS}}\) can associate with focal adhesion proteins in normal and BCR/ABL transformed cells. Immunoprecipitates of cell lysates (Control I.P., \(20 \times 10^6\) 32D.p210BCR/ABL 26 cells, first lane) with control antibody against \(\gamma\)-interferon, and immunoprecipitates of cell lysates (I.P., \(20 \times 10^6\) cells, last four lanes) with various antibodies (anti-tensin, anti-p125\(^{\text{FAK}}\), anti-vinculin, and anti-paxillin) were processed as described under “Materials and Methods” and applied to a gradient SDS-PAGE gel (6–12%) and transferred to Immobilon-P membrane. The membranes were immunoblotted with anti-p130\(^{\text{CAS}}\). The last four lanes (32D(−)), 32Dc13 stimulated cells with interleukin-3 (32D(+)), 32Dc13 stimulated cells and 32D.p210BCR/ABL 26 cells (32D p210), and K562 cells.

In hematopoietic cells, we have tried to determine if they have a similar association with the recently cloned Crk-associated substrate p130\(^{\text{CAS}}\) (14, 15). Our results indicate that p130\(^{\text{CAS}}\) is tyrosine phosphorylated, and associates with CRKL, but not c-CRK in BCR/ABL transformed cell lines and samples from patients with CML and Ph\(^{+}\) ALL. The timing of association between p130\(^{\text{CAS}}\) and BCR/ABL to CRKL has not been shown in this study and further experiments will be needed.

It is of interest that p130\(^{\text{CAS}}\) is not significantly tyrosine phosphorylated in K562 cells and the reasons are quite unclear (2, 29). There is a possibility that K562 cells have other abnormalities which prevent p130\(^{\text{CAS}}\) from becoming phosphorylated. Classically, there is heterogeneity of tyrosine phosphorylation of various proteins in CML primary cells. This heterogeneity could also involve the tyrosine phosphorylation of p130\(^{\text{CAS}}\) by BCR/ABL. It has also been shown that K562 cells lack 32 integrins and this somehow is related to p130\(^{\text{CAS}}\) phosphorylation (39). Eventually, further experiments will need to be performed to explore the differences and localization of p130\(^{\text{CAS}}\).

p130\(^{\text{CAS}}\) was initially identified as a prominent tyrosine-phosphorylated substrate of the oncoproteins v-src and v-Crk (17, 40). It has also been shown that p130\(^{\text{CAS}}\) is tyrosine phosphorylated in response to adhesion and cross-linking of \(\beta\) integrins (17, 18, 20, 21). p130\(^{\text{CAS}}\) has been shown to associate with the focal adhesion proteins p125\(^{\text{FAK}}\) and tensin \(\text{in vitro}\) and \(\text{in vivo}\) (19, 41). p130\(^{\text{CAS}}\) may be important in transformation by ornithine decarboxylase (42). It has been shown that c-Abl may phosphorylate p130\(^{\text{CAS}}\) \(\text{in vitro}\). In normal cells, p130\(^{\text{CAS}}\) is localized to both the cytoplasm and the nucleus; whereas in v-src and v-crk transformed fibroblasts, it is primarily localized to the focal adhesion (14, 17, 19).

p130\(^{\text{CAS}}\) was originally cloned from 3Y1 fibroblasts transformed with v-crk (14, 15). There are two major tyrosine phosphorylated proteins in v-crk transformed 3Y1 cells: paxillin and p130\(^{\text{CAS}}\) (43). Using isolated protein and peptide sequencing, the cDNA encoding p130\(^{\text{CAS}}\) has been characterized. p130\(^{\text{CAS}}\) contains an NH\(_2\)-terminal SH3 domain which has been shown to bind to the proline-rich portion in the COOH terminus of p125\(^{\text{FAK}}\). In the middle of the molecule is the “substrate domain” with nine Tyr-Asp-Pro motifs optimum for binding the c-Crk-SH2 domain (14). Mayer et al. (44) have shown that the c-Abl-SH2 domain binds to the substrate domain of p130\(^{\text{CAS}}\) \(\text{in vitro}\). Lo et al. (19) have shown that the SH2 domain of tensin is also capable of binding p130\(^{\text{CAS}}\) in v-src transformed cells. We show here that the CRKL-SH2 domain binds directly to the substrate domain of p130\(^{\text{CAS}}\).

We examined the association of p130\(^{\text{CAS}}\) with focal adhesion proteins in normal and BCR/ABL transformed hematopoietic cells. There was a constitutive association of p125\(^{\text{FAK}}\) and paxillin with p130\(^{\text{CAS}}\). Interestingly, the focal adhesion protein tensin associated with p130\(^{\text{CAS}}\) in normal hematopoietic cells, but not in BCR/ABL transformed cells. Tensin is an SH2 containing protein which has three actin-binding domains, and is believed to be involved in linking integrins to the actin cytoskeleton (45). Tensin also is tyrosine phosphorylated in response to various mitogenic signals (36). In this study, we have shown that tensin and p130\(^{\text{CAS}}\) form a complex in normal cells and that binding is disrupted in BCR/ABL transformed cells. The reason for this possibility could be that tensin and/or p130\(^{\text{CAS}}\) could be located in different compartments, either of these molecules could be bound to another protein thereby not interacting with each other, or the tyrosine phosphorylation of either of these molecules could potentially inhibit their interactions. The alteration in binding between p130\(^{\text{CAS}}\) and tensin could be an important step toward BCR/ABL transformation, and this is currently being investigated.

In summary, we have shown that p130\(^{\text{CAS}}\) is expressed in hematopoietic cells, it is tyrosine phosphorylated in response to BCR/ABL, and associates with the unique adapter protein CRKL as shown by immunoprecipitation. p130\(^{\text{CAS}}\) also constitutively associates with focal adhesion proteins such as paxillin and p125\(^{\text{FAK}}\). However, p130\(^{\text{CAS}}\) associates with tensin in normal but not BCR/ABL expressing cells. It is known that CML progenitor cells have abnormalities of adhesion through \(\beta\) integrins and potentially p130\(^{\text{CAS}}\) could be involved in mediating these abnormalities.

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REFERENCES
1. Andoniou, C. E., Thien, C. B. F., and Langdon, W. Y. (1994) EMBO J. 13, 4515–4521
2. de Jong, R., ten Hoeve, J., Heisterkamp, N., and Groffen, J. (1995) J. Biol. Chem. 270, 21468–21471
3. Matulonis, U., Salgia, R., Okuda, K., Druker, B., and Griffin, J. (1993) Exp. Hematol. 21, 1460–1466
4. Salgia, R., Brunkhorst, B., Pisick, E., Li, J. L., Lo, S. H., Chen, L. B., and Griffin, J. D. (1995) Oncogene 11, 1149–1155
5. ten Hoeve, J., Arlinghaus, R. B., Guo, J. Q., Heisterkamp, N., and Groffen, J. (1994) Blood 84, 1731–1736
6. Nichols, G. L., Raines, M. A., Vera, J. C., Lacomis, L., Tempst, P., and Golde, D. W. (1994) Blood 84, 2912–2918
7. Oda, T., Henney, C., Hagara, J. R., Okuda, K., Griffin, J. D., and Druker, B. J. (1994) J. Biol. Chem. 269, 22925–22928
8. Mayer, B. J., and Hanafusa, H. (1990) J. Virol. 64, 3581–3589
9. ten Hoeve, J., Morris, C., Heisterkamp, N., and Groffen, J. (1995) Oncogene 8, 2469–2474
10. Matsuda, M., Tanaka, S., Nagata, S., Kojima, A., Kurata, T., and Shibuya, M. (1992) Mol. Cell. Biol. 12, 3482–3489
11. Matsuda, M., Mayer, B. J., and Hanafusa, H. (1991) Mol. Cell. Biol. 11, 1607–1613
12. Matsuda, M., Reichman, C. T., and Hanafusa, H. (1992) J. Virol. 66, 115–121
13. Ren, R., Ye, Z. S., and Baltimore, D. (1994) Genes Dev. 8, 783–789
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14. Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Mano, H., Yazaki, Y., and Hirai, H. (1994) *EMBO J.* 13, 3748–3756

15. Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Nishida, J., Yazaki, Y., and Hirai, H. (1994) *J. Biol. Chem.* 269, 32740–32746

16. Nakamoto, T., Sakai, R., Ozawa, K., Yazaki, Y., and Hirai, H. (1996) *J. Biol. Chem.* 271, 8959–8965

17. Kanner, S. B., Reynolds, A. B., Wang, H. C., Vines, R. R., and Parsons, J. T. (1991) *EMBO J.* 10, 1689–1698

18. Petch, L. A., Bockholt, S. M., Bouton, A., Parsons, J. T., and Burridge, K. (1995) *J. Cell Sci.* 108, 1371–1379

19. Lo, S. H., Weisberg, E., and Chen, L. B. (1994) *Bioessays* 16, 817–823

20. Nojima, Y., Morino, N., Mimura, T., Hamasaki, K., Furuya, H., Sakai, R., Sato, T., Tachibana, K., Morimoto, C., Yazaki, Y., and Hirai, H. (1995) *J. Biol. Chem.* 270, 15398–15402

21. Vuori, K., and Ruoslahti, E. (1995) *J. Biol. Chem.* 270, 22259–22262

22. Sattler, M., Salgia, R., Okuda, K., Uemura, N., Durstin, M., Pisick, E., Xu, G., Li, J.-L., Prasad, K., and Griffin, J. (1996) *Oncogene* 12, 839–846

23. Griffin, J. D., Spertini, O., Ernst, T. J., Belvin, M. P., Levine, H. B., Kanakura, Y., and Tedder, T. F. (1990) *J. Immunol.* 145, 576–584

24. Salgia, R., Li, J.-L., Lo, S. H., Brunekhorst, B., Kansas, G. S., Sobhany, E. S., Sun, Y., Pisick, E., Hallek, M., Ernst, T., Tantravahi, R., Chen, L. B., and Griffin, J. D. (1995) *Exp. Hematol.* 23, 1040–1048

25. ten Hoeve, J., Kaartinen, V., Fietroz, T., Haataja, L., Vonck, J., Heisterkamp, N., and Groffen, J. (1994) *Cancer Res.* 54, 2563–2567

26. Salgia, R., Uemura, N., Okuda, K., Li, J.-L., Pisick, E., Sattler, M., de Jong, R., Drucker, B., Heisterkamp, N., Chen, L. B., Groffen, J., and Griffin, J. D. (1995) *J. Biol. Chem.* 270, 29145–29150

27. Larson, R., and Springer, T. (1990) *Cell* 60, 181–217

28. Birge, R. B., Fajardo, J. E., Mayer, B. J., and Hanafusa, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 10678–10682

29. Arvieux, M., Paasinensohns, A., Hirai, H., Andersson, L. C., and Haltia, E. (1995) *Cell* 61, 1535–1543

30. Surin, T., Kanner, S. B., Reynolds, A. B., Wang, H. C., Vines, R. R., and Parsons, J. T. (1991) *EMBO J.* 10, 1689–1698

31. Kanner, S. B., Reynolds, A. B., Wang, H. C., Vines, R. R., and Parsons, J. T. (1991) *EMBO J.* 10, 1689–1698

32. Petch, L. A., Bockholt, S. M., Bouton, A., Parsons, J. T., and Burridge, K. (1995) *J. Cell Sci.* 108, 1371–1379

33. Lo, S. H., Weisberg, E., and Chen, L. B. (1994) *Bioessays* 16, 817–823

34. Nojima, Y., Morino, N., Mimura, T., Hamasaki, K., Furuya, H., Sakai, R., Sato, T., Tachibana, K., Morimoto, C., Yazaki, Y., and Hirai, H. (1995) *J. Biol. Chem.* 270, 15398–15402

35. Vuori, K., and Ruoslahti, E. (1995) *J. Biol. Chem.* 270, 22259–22262

36. Sattler, M., Salgia, R., Okuda, K., Uemura, N., Durstin, M., Pisick, E., Xu, G., Li, J.-L., Prasad, K., and Griffin, J. (1996) *Oncogene* 12, 839–846

37. Griffin, J. D., Spertini, O., Ernst, T. J., Belvin, M. P., Levine, H. B., Kanakura, Y., and Tedder, T. F. (1990) *J. Immunol.* 145, 576–584

38. Salgia, R., Li, J.-L., Lo, S. H., Brunekhorst, B., Kansas, G. S., Sobhany, E. S., Sun, Y., Pisick, E., Hallek, M., Ernst, T., Tantravahi, R., Chen, L. B., and Griffin, J. D. (1995) *Exp. Hematol.* 23, 1040–1048

39. ten Hoeve, J., Kaartinen, V., Fietroz, T., Haataja, L., Vonck, J., Heisterkamp, N., and Groffen, J. (1994) *Cancer Res.* 54, 2563–2567

40. Salgia, R., Uemura, N., Okuda, K., Li, J.-L., Pisick, E., Sattler, M., de Jong, R., Drucker, B., Heisterkamp, N., Chen, L. B., Groffen, J., and Griffin, J. D. (1995) *J. Biol. Chem.* 270, 29145–29150

41. Larson, R., and Springer, T. (1990) *Cell* 60, 181–217

42. Birge, R. B., Fajardo, J. E., Mayer, B. J., and Hanafusa, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 10678–10682

43. Arvieux, M., Paasinensohns, A., Hirai, H., Andersson, L. C., and Haltia, E. (1995) *Cell* 61, 1535–1543

44. Birge, R. B., Fajardo, J. E., Reichman, C., Shoelson, S. E., Sengyang, Z., Cantley, L. C., and Hanafusa, H. (1993) *Mol. Cell. Biol.* 13, 4648–4656

45. Mayer, B. J., Hirai, H., and Sakai, R. (1995) *Curr. Biol.* 5, 296–305

46. Lo, S. H., Janmey, P. A., Hartwig, J. H., and Chen, L. B. (1994) *J. Cell Biol.* 123, 1067–1075