Interaction of Hic-5, A Senescence-related Protein, with Focal Adhesion Kinase*

(Received for publication, March 31, 1998, and in revised form, July 9, 1998)

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Hydrogen peroxide-inducible clone (Hic)-5 is induced during the senescent process in human fibroblasts, and the overexpression of Hic-5 induces a senescence-like phenotype. Structurally, Hic-5 and paxillin, a 68-kDa cytoskeletal protein, share homology such as the LD motifs in the N-terminal half and the LIM domains in the C-terminal half. Here we show that Hic-5 binds to focal adhesion kinase (FAK) by its N-terminal domain, and is localized to focal adhesions by its C-terminal LIM domains. However, Hic-5 is not tyrosine phosphorylated either by the coexpressed FAK in COS cells or by integrin stimulation in 293T cells. Furthermore, overexpression of Hic-5 results in a decreased tyrosine phosphorylation of paxillin. These findings suggest that putative functions of Hic-5 are the recruitment of FAK to focal adhesions and a competitive inhibition of tyrosine phosphorylation of paxillin.

Integrin-mediated cell adhesion induces various biological events such as cell proliferation, survival, migration, cytokine production, and cytoskeleton reorganization (1–7). Despite the significance of these events, signal transduction pathways of integrins have not been fully understood. One of the approaches for integrin-mediated signal transduction is to study the proteins that are localized to focal adhesions.

Focal adhesions are the sites where cells attach to substrata via integrin-extracellular matrix binding (7, 8). Various cytoskeletal proteins and signaling molecules such as focal adhesion kinase (FAK),1 paxillin, vinculin, talin, p130Cas, and tensin, are accumulated in the cytoplasmic part of focal adhesions (7–10). More interestingly, focal adhesions are strongly stained by immunohistochemical analysis with anti-phosphotyrosine mAb (7, 10). Several proteins localized to focal adhesions such as FAK, paxillin, and p130Cas, are tyrosine phosphorylated following integrin stimulation (7, 9, 10). When tyrosine phosphorylated, these proteins bind to signaling molecules such as Crk, Nck, GRB2, Src, which contain Src homology (SH) 2 domain (7, 10). Paxillin, a 68-kDa cytoskeletal protein, is one of these focal adhesion proteins (11–13) and has two functions in integrin-mediated signal transduction. First, paxillin appears to be the major entity that recruits two binding proteins, FAK, and vinculin (11–15), to focal adhesions (16, 17). Second, paxillin is tyrosine phosphorylated following integrin stimulation in a FAK-dependent manner, and then binds to the Crk SH2 domain (15, 18). Thus, paxillin is involved in recruitment of FAK to focal adhesions, tyrosine phosphorylated by FAK and/or a kinase(s) that is associated with FAK, and then works as a docking protein in integrin-mediated signal transduction.

Hydrogen peroxide-inducible clone (hic)-5 was initially identified as a gene which was induced by transforming growth factor β1 or by H2O2 (19). The expression of hic-5 mRNA was augmented in the in vitro senescent process of human diploid fibroblasts (19). The forced overexpression of Hic-5 induced growth retardation, senescence-like morphology such as the enlarged and flattened morphology, and the increased expression of p21/WAF1/Cip1/sdi1 and extracellular matrix-related proteins such as fibronectin (FN), collagen, and collagenase (20). These findings suggest that Hic-5 is involved in very interesting function in the senescent process and in transforming growth factor β1-mediated signal transduction. However, the nature of Hic-5 function has not been clarified yet. Structurally, Hic-5 contains four LIM domains in the C-terminal half and LD domains in the N-terminal half (19, 21). These domains are conserved in paxillin, and the four LIM domains of Hic-5 and paxillin share 62% homology (17, 19–21). These structural homology with paxillin suggested that Hic-5 is localized to focal adhesions and involved in integrin-mediated signal transduction. Recently, Matsuya et al. (21) reported that Hic-5 bound to Cak β, a FAK-related tyrosine kinase, and was localized to focal adhesions.

In this study, we demonstrate that Hic-5 is localized to focal adhesions by its LIM domains and binds to the FAK C-terminal domain by its N-terminal half. However, unlike paxillin, Hic-5 is not tyrosine phosphorylated by FAK and by integrin stimulation, suggesting that Hic-5 and paxillin have a different function in integrin-mediated protein tyrosine phosphorylation.

MATERIALS AND METHODS

Cell Culture and Transfection—COS1, 293T, Swiss 3T3, 3Y1, and SR-3Y1 cells were described previously (16, 22, 23). Bosc 23 was obtained from ATCC (24). Cells were cultured in RPMI 1640 or Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum and 20 µg/ml gentamicin. Plasmid DNAs were transfected into cells using LipofectAMINE (Life Technologies, Inc.) following the manufacturer’s protocol. Usually, 4 µg of DNA were used for a 10-cm dish. In infection studies, Swiss 3T3 cells were incubated in the super-
natant of the pBABE-transfected Bosc23 cells with 4 μg/ml hexa-
dimethrine bromide (Sigma) overnight (24). For FN stimulation, cells
were trypsinized, washed in Dulbecco’s modified Eagle’s medium, and
then incubated 1 h on a dish coated with human plasma FN (Life
Technologies, Inc.) (23).

**Plasmid DNAs—**The oligonucleotides of the c-Myc epitope
(MEQLISEEDEL) was jointed to the cDNAs of mouse hic-5 and human
paxillin by polymerase chain reaction-mediated method (23). Human
paxillin cDNA was kindly provided by Dr. Ravi Salgia and Dr. James D.
Griffin (Dana-Faber Cancer Institute, Boston, MA). c-Myc-tagged hic-5
and paxillin were inserted into an expression vector, pMT3. Hemagglu-
tinin (HA) epitope-tagged wild-type FAK and mutants were described
elsewhere (23). The N-terminal half of the hic-5 cDNA was inserted into
pGEX (Pharmacia LKB, Uppsala, Sweden). The other plasmids used to
generate the glutathione S-transferase (GST) fusion proteins of FAK
were described before (16). The DNA fragment coding enhanced green
fluorescent protein (EGFP, CLONTECH, Palo Alto, CA) was jointed to
the hic-5 cDNA and inserted into a retrovirus vector pBABE (25).

**Antibodies, Precipitations, and Immunoblotting—**The GST fusion
protein of the Hic-5 N-terminal half (residues 2 to 249) was immunized
to a rabbit to generate anti-Hic-5 polyclonal antibody (pAb). Rabbit
anti-Hic-5 pAb was purified from anti-sera with protein A-conjugated
beads (Pharmacia LKB) for immunoblotting and immunoprecipitation,
and was affinity purified with the GST-Hic-5 fusion protein for immu-
nohistochemistry. Cells were lysed in 1% Nonidet P-40 lysis buffer as
described (16). Proteins were precipitated with Ab plus protein-A con-
jugated beads or with glutathione beads (Pharmacia LKB) conjugated
with GST-FAK fusion protein. Cellular lysates and precipitates were
fractionated by SDS-polyacrylamide gel electrophoresis and electro-
transferred onto nitrocellulose membranes. Immunoblotting was per-
formed with a primary Ab, horseradish peroxidase-conjugated second-
ary Ab (Amersham Corp.), and chemiluminescence reagent (NEN Life
Science Products Inc.), or with 125I-labeled anti-phosphotyrosine
mAb (4G10, Upstate Biotechnology, Inc.) as described (22, 23). The
relative intensity of each band was digitalized by scanning films with
Alpha Imager 2000 (Alpha Innotech Corp., San Leandro, CA).

**Immunohistochemistry—**3T1 cells were incubated on glass coverslips
for 2 days. Cells were washed with phosphate-buffered saline (PBS),
immersed, and fixed in the fixation solution (3.7% paraformaldehyde,
0.1% Triton X-100, in phosphate-buffered saline), and immunostained
with primary Ab and then fluorescein-conjugated anti-mouse or anti-
rabbit Ab (KPL, Gaithersburg, MD). After infection of retroviruses, Swiss
3T3 cells were cultured on glass coverslips for 2 days, and then washed
and fixed. Subcellular localization of proteins were analyzed with flu-
oromicroscopy (Axioskop; Carl Zeiss Inc., Thornwood, NY) as described
(16).

**RESULTS**

We reported FAK-paxillin interaction and the paxillin-medi-
ated focal adhesion targeting of FAK (16). Because Hic-5 has a
similar structure as paxillin, we studied FAK-Hic-5 interaction
and the subcellular localization of Hic-5. To define the FAK-
Hic-5 interaction, HA epitope-tagged FAK was coexpressed
with c-Myc epitope-tagged Hic-5 or paxillin in COS1 cells.
Then, Hic-5 or paxillin was immunoprecipitated with anti-c-
Myc mAb, and the coprecipitation of FAK was investigated by
immunoblotting with anti-HA mAb. As shown in Fig. 1A, HA-

**Fig. 1. Binding of Hic-5 to FAK.** A, co-immunoprecipitation of FAK
with Hic-5. HA-tagged FAK and c-Myc-tagged Hic-5 or paxillin
were coexpressed in COS1 cells by the transfection of plasmids. The binding
of FAK to Hic-5 or paxillin was detected by coprecipitation with Hic-5 or
paxillin using immunoprecipitation with anti-c-Myc-tag mAb (9E10, Oncogene Science Inc., Manhasset, NY) and immunoblotting with anti-
HA tag mAb (12CA5, Boehringer Mannheim). Expression of Hic-5 and
paxillin was shown by anti-c-Myc blot. A similar result using HA-tagged
FAK-C-terminal domain (FAK-CT) instead of wild-type FAK was
shown at the bottom. FAK-CT contains FAK residues 707–1052. B, the
FAK-binding site of Hic-5. c-Myc-tagged wild-type, N-terminal half, and
C-terminal half of Hic-5 were expressed in COS1 cells. Cellular lysates
were precipitated with GST fusion protein of FAK-CT (GST-FAK-CT)
and analyzed by immunoblotting with anti-c-Myc mAb. C, the binding
specificity of Hic-5 to FAK. c-Myc-tagged Hic-5 and paxillin were
expressed in COS1 cells, precipitated with various mutants of GST-FAK
(16), and then analyzed by immunoblotting with anti-c-Myc mAb. Each
substitution mutant contains a single amino acid substitution in GST-
FAK (903–1052). The lanes of lysates were shown as (-).
by immunoblotting with anti-FAK mAb (10G2, Ref. 16). Lysates of 3Y1, SR-3Y1, 293T, Swiss 3T3, COS1, and COS1 cells that expressed c-Myc-tagged Hic-5 were analyzed by immunoblotting with rabbit anti-Hic-5 pAb. Then, the same blot was analyzed with anti-paxillin mAb (Transduction Laboratories, Lexington, KY). The size of paxillin was indicated in anti-Hic-5 blot. Cellular lysate corresponding to one-fifth of subconfluent cells in a 10-cm dish was loaded on a lane in each cell line. In Hic-5 transfected COS1 cells, lysate of 1/50 of the 10-cm dish was loaded. B, in vivo binding of Hic-5 to FAK. Endogenous Hic-5 protein was immunoprecipitated with anti-Hic-5 pAb from the lysate of 293T cells, and the coprecipitated FAK was detected by immunoblotting with anti-FAK mAb (10G2, Ref. 16).

tagged wild-type FAK was coimmunoprecipitated with Hic-5 and paxillin. A similar result was obtained when HA-tagged FAK C-terminal domain (FAK-CT) was coexpressed with Hic-5 or paxillin. Therefore, like paxillin, Hic-5 can bind to the C-terminal domain of FAK.

Paxillin binds to the C-terminal domain of FAK by its N-terminal half. To determine the FAK-binding site of Hic-5, the c-Myc-tagged N-terminal and C-terminal halves of Hic-5 were expressed in COS1 cells and precipitated with the GST fusion protein of the FAK C-terminal domain (GST-FAK-CT). As shown in Fig. 1B, the wild-type and N-terminal half of Hic-5 were precipitated with GST-FAK-CT, whereas the C-terminal half of Hic-5 was not precipitated with GST-FAK-CT. Taken together, the N-terminal domain of Hic-5 can bind to the FAK-CT. We next defined the binding specificity of FAK to Hic-5 and paxillin. c-Myc-tagged Hic-5 and paxillin were expressed in COS1 cells, precipitated with various mutants of GST-FAK, and detected with immunoblotting with anti-c-Myc mAb. As shown in Fig. 1C, both Hic-5 and paxillin were precipitated with GST-FAK (903–1052) and its substitution mutants, K933E and Q1040G. However, neither protein were precipitated with GST and GST-FAK mutants, V928G and L1034S. These results indicate that Hic-5 and paxillin share a very similar FAK binding specificity.

For further analysis, we developed rabbit anti-Hic-5 polyclonal Ab (pAb) and used this antibody to detect endogenous Hic-5 protein. As shown in Fig. 2A, a 54-kDa peptide was detected by this anti-Hic-5 pAb in the lysate of rat fibroblast, 3Y1. Since this peptide was migrated to the same size as c-Myc-tagged mouse Hic-5 that was expressed in COS-1 cells by transfection, this 54-kDa peptide represented endogenous Hic-5 in 3Y1 cells. A protein with a similar molecular weight was detected in mouse fibroblasts, Swiss 3T3 cells, although the expression level of this protein was lower than in 3Y1. In Src-transformed 3Y1 cells, a slightly slower migrated protein in addition to this 54-kDa protein was detected. In contrast, a 56-kDa peptide was detected by this anti-Hic-5 pAb in the lysate of human kidney-derived 293T cells, and in African green monkey kidney-derived COS1 cells. p68 paxillin, a Hic-5 related protein, was not detected by this anti-Hic-5 pAb, although paxillin was expressed in these cells as shown by reblotting with anti-paxillin mAb.

Using this anti-Hic-5 pAb, we studied the in vivo association of Hic-5 and FAK. Endogenous Hic-5 was immunoprecipitated by anti-Hic-5 pAb from the lysate of 293T cells. Then, precipitated were analyzed by immunoblotting with anti-FAK mAb. As shown in Fig. 2B, endogenous FAK was coprecipitated with endogenous Hic-5, indicating the FAK-Hic-5 interaction in vivo.

For further analysis, we studied the subcellular localization of Hic-5. Since FAK is localized to focal adhesions, Hic-5 should be localized to focal adhesions if Hic-5 binds to FAK in vivo. We determined the subcellular localization of Hic-5 in 3Y1 cells by immunohistochemical analysis with anti-Hic-5 pAb. As shown in Fig. 3A, Hic-5 was demonstrated as rod-shaped staining in cytoplasm, mostly in the periphery of a cell, with a direction of peripheral to center of a cell (arrows). This staining of Hic-5 was similar as the staining of vinculin which was shown as typical focal adhesions, and was located at the identical sites with vinculin in double staining, indicating that Hic-5 is localized to focal adhesions in 3Y1 cells. Next, we wished to determine the Hic-5 domain which is responsible for the focal adhesion targeting. Since Brown et al. (17) reported that paxillin is localized to focal adhesions by its C-terminal LIM domains, it is likely that the Hic-5 C-terminal LIM domains are essential for the focal adhesion targeting of Hic-5. To determine the focal adhesion targeting domain of Hic-5, we expressed EGFP-tagged wild-type Hic-5 and its deletion mutants in Swiss 3T3 cells and analyzed their subcellular localization under fluorescent microscopy. As shown in Fig. 3B, the EGFP-tagged wild-type and C-terminal half of Hic-5 were detected at focal adhesions, whereas EGFP alone and the EGFP-tagged Hic-5 N-terminal half were localized diffusely and were not detected at focal adhesions. Thus, Hic-5 is localized to focal adhesions by its C-terminal LIM domains. However, the EGFP-tagged wild-type and N-terminal half of Hic-5 were localized outside of nuclei, whereas EGFP alone and the EGFP-tagged C-terminal domain were localized both inside and outside of nuclei. Therefore, the N-terminal domain of Hic-5 appears to be involved in the cytoplasmic localization of Hic-5.

Thus, Hic-5 and paxillin share not only structural homology, but also several characteristics such as binding activity to FAK and subcellular localization to focal adhesions. However, Hic-5 does not contain several tyrosine residues in paxillin, which are phosphorylated by integrin stimulation and putative binding sites for the Crk SH2 domain. Therefore, we next studied whether Hic-5 is tyrosine phosphorylated by integrin stimulation or not. c-Myc-tagged Hic-5 and paxillin were coexpressed with HA-tagged FAK, immunoprecipitated, and analyzed by immunoblotting with anti-phosphotyrosine mAb (anti-Tyr(P)). As shown in Fig. 4A, paxillin was tyrosine phosphorylated when coexpressed with FAK wild-type or Y397F mutant. Since paxillin was not tyrosine phosphorylated when coexpressed with FAK-kinase negative, this phosphorylation of paxillin was dependent on the kinase activity of the coexpressed FAK.
rosine phosphorylation of paxillin by FAK-Y397F was less compared with that by wild-type FAK. These results were consistent with the reports of Schaller and Parsons (15) and Bellis et al. (18), and indicated tyrosine phosphorylation of paxillin by FAK and a FAK-associated kinase. Unlike paxillin, Hic-5 was not tyrosine phosphorylated when coexpressed with FAK. These data strongly suggest that Hic-5 is not a substrate of FAK, whereas paxillin is a substrate of both FAK and a tyrosine kinase(s) that binds to phosphorylated FAK-Y397.

Next, we investigated tyrosine phosphorylation of Hic-5 by integrin stimulation. c-Myc-tagged Hic-5 or paxillin were expressed in 293T cells by transfection. These 293T cells were detached from substrata, incubated on the plates coated with FN or poly-L-lysine (Sigma), and then lysed in 1% Nonidet P-40 lysis buffer. Hic-5 and paxillin were immunoprecipitated from cellular lysates with anti-c-Myc mAb and analyzed by immunoblotting with anti-phosphotyrosine mAb. As shown in Fig. 4B, paxillin was tyrosine phosphorylated following integrin stimulation, whereas no increased tyrosine phosphorylation of Hic-5 was observed upon FN stimulation. We also analyzed integrin-mediated tyrosine phosphorylation of endogenous Hic-5 and paxillin in 293T cells. As shown in Fig. 4C, paxillin was tyrosine phosphorylated following FN stimulation. In contrast, we observed no increased tyrosine phosphorylation of endogenous Hic-5, further indicating that Hic-5 is not tyrosine phosphorylated following integrin stimulation.

The expression of Hic-5 is augmented in a senescent process in human fibroblasts. To elucidate a putative function of Hic-5, we studied integrin-mediated tyrosine phosphorylation of paxillin in the presence or absence of Hic-5. c-Myc-tagged paxillin and Hic-5 were coexpressed in 293T cells. Then, cells were detached and stimulated by the plates coated with FN. Paxillin and Hic-5 were immunoprecipitated with anti-c-Myc mAb and analyzed by immunoblotting with anti-phosphotyrosine mAb. As shown in Fig. 4D, FN-induced tyrosine phosphorylation of paxillin was decreased by the coexpression of Hic-5 in a dose-dependent manner. This result suggests that overexpression of Hic-5 inhibits the signal from tyrosine-phosphorylated paxillin.

DISCUSSION

In this article, we have demonstrated that Hic-5, a senescence-related protein, is a cytoskeletal protein localized to focal adhesions. Similar to a related protein, paxillin, Hic-5 is localized to focal adhesions by its C-terminal LIM domains and binds to FAK by its N-terminal domain. Therefore, Hic-5 appears to be involved in the recruitment of FAK to focal adhesions and involved in integrin signal transduction.

However, unlike paxillin, Hic-5 is not tyrosine phosphorylated by integrin stimulation, or by the coexpression of FAK. Furthermore, overexpression of Hic-5 inhibited integrin-mediated tyrosine phosphorylation of paxillin. Gilmore and Romer (26) reported that microinjection of GST-FAK-CT resulted in reduced focal adhesion phosphotyrosine and reduced DNA synthesis. GST-FAK-CT contains the focal adhesion targeting domain, but does not contain a kinase domain. Therefore, GST-FAK-CT inhibits tyrosine phosphorylation of focal adhesion proteins by preventing endogenous FAK localization to focal adhesions and the binding of FAK to focal adhesion proteins. These findings strongly suggest that prevention of tyrosine phosphorylation of focal adhesion proteins, such as paxillin, inhibits cell proliferation.

The phosphotransfer sites of paxillin are putative binding sites for the SH2 domain of Crk, an oncogenic adapter protein (15, 18, 27). Crk is composed of SH2 and SH3 domains (27). Crk binds to tyrosine-phosphorylated proteins by its SH2 domain and binds to the effector proteins by its SH3 domains (28, 29). Thus, Crk is involved in the recruitment of signaling molecules,
such as C3G and SOS, to tyrosine-phosphorylated docking proteins (28, 29). Since paxillin is localized at focal adhesions, the binding of Crk to tyrosine-phosphorylated paxillin results in the recruitment of these signaling molecules to focal adhesions that are localized close to cytoplasmic membrane. C3G and SOS are guanine nucleotide exchange factors for Rap1A/Rev-1 and Ras, respectively (30, 31). These small GTPases are involved in the regulation of mitogen-activated protein (MAP) kinases (32, 33). Inhibition studies using Crk mutants showed that Crk is involved in the regulation of MAP kinase (34, 35). v-Crk and C3G are also involved in the activation of c-Jun N-terminal kinase, another MAP kinase family kinase (36). Taken together, tyrosine phosphorylation of paxillin may be involved in the activation of MAP kinase family kinases through the recruitment of Crk. Since activation of MAP kinase family kinases regulates gene expression and cell proliferation, the overexpression of Hic-5 may induce growth retardation by blocking activation of MAP kinases.

Inhibition of tyrosine phosphorylation of focal adhesion proteins causes alterations in cell shape and a decrease in cell migration. FAK knock-out or the overexpression of FAK-CT resulted in enlarged focal adhesions and reduced cell motility (37, 38). More directly, Klemke et al. (39) reported that the binding of Crk to another focal adhesion protein, Crk-associated substrate (p130Cas), induces cell migration and membrane ruffling (39). We have observed a similar phenomenon in Cas-L transfected cells.2 These findings suggest that the recruitment of Crk to paxillin can induce membrane ruffling and cell migration. Therefore, parts of senescent phenotypes, such as the enlarged and flattened morphology of cells, may be caused by the inhibition of tyrosine phosphorylation of paxillin.

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2 K. Tachibana, K. Kamiguchi, T. Azuma, and C. Morimoto, unpublished data.

**FIG. 4. Analysis of tyrosine phosphorylation of Hic-5.** A, Hic-5 was not tyrosine phosphorylated by the coexpression of FAK. c-Myc-tagged Hic-5 and paxillin were coexpressed with HA-tagged wild-type, Y397F, or K454R (kinase negative) FAK in COS1 cells. Tyrosine phosphorylation of Hic-5 and paxillin was analyzed by immunoprecipitation with anti-c-Myc mAb and immunoblotting with anti-phosphotyrosine mAb (anti-Tyr(P)). The amounts of the precipitated Hic-5, paxillin, and coprecipitated FAK were demonstrated by immunoblotting. B, Hic-5 was not tyrosine phosphorylated following integrin stimulation. c-Myc-tagged Hic-5 and paxillin were expressed in 293T cells. Cells were detached, and then reattached to plates coated with FN or poly-L-lysine (PLL, Sigma). Hic-5 and paxillin were isolated by immunoprecipitation with anti-c-Myc mAb and analyzed by immunoblotting with anti-Tyr(P). C, endogenous Hic-5 was not tyrosine phosphorylated following integrin stimulation. Endogenous Hic-5 or paxillin were immunoprecipitated with anti-Hic-5 pAb or anti-paxillin mAb from the lysates of FN- or poly-L-lysine-stimulated 293T cells, and analyzed by immunoblotting with anti-Tyr(P). Both blots were from one membrane, and the anti-Tyr(P) blots were of the same exposure. D, inhibition of integrin-mediated tyrosine phosphorylation of paxillin by the co-expression of Hic-5. pMT3-c-Myc-tagged paxillin (0.25 μg/10 cm-plate) was co-transfected with various amounts (0–10 μg) of pMT3-c-Myc-tagged hic-5 into 293T cells. Transfected cells were detached, incubated on FN- or poly-L-lysine-coated plates, and lysed. c-Myc-tagged paxillin was immunoprecipitated and subjected to the analysis with anti-Tyr(P).
In summary, our study, along with the report of Matsuya et al. (21), addressed that Hic-5 has two distinct functions in integrin-mediated signal transduction, the recruitment of FAK and Cak β to focal adhesions and the inhibition of tyrosine phosphorylation of paxillin. Since integrin-mediated cell adhesion induces cell proliferation and survival, overexpression of Hic-5 may induce senescence-like phenotypes in human diploid fibroblasts by modulating integrin-mediated signal transduction.

We also found that paxillin was tyrosine phosphorylated by the coexpression of FAK-Y397F in COS1 cells. Since Src family tyrosine kinases do not bind to FAK-Y397F (40), this result indicates that FAK can directly phosphorylate paxillin. We reported previously that Crk-associated substrate (p130Cas) and Cas-L are substrates of FAK (23). Tyrosine-phosphorylated Cas family proteins such as paxillin and Cas-L are substrates of FAK (21), addressed that Hic-5 has two distinct functions in integrin-mediated signal transduction, the recruitment of FAK to focal adhesions and the inhibition of tyrosine phosphorylation of paxillin. Since integrin-mediated cell adhesion induces cell proliferation and survival, overexpression of Hic-5 may induce senescence-like phenotypes in human diploid fibroblasts by modulating integrin-mediated signal transduction.

Acknowledgments—We thank Dr. Ravi Salgia and Dr. James D. Griffin for paxillin cDNA and thank Dr. Martin E. Hemler (Dana-Faber Cancer Institute, Boston, MA) for fluoromicroscopy. We thank Drs. Rumiko and Terukatsu Sasaki (Sapporo Medical University, Sapporo, Japan) and Dr. Shiera Thomas (Beth Israel and Deaconess Hospital, Boston, MA) for helpful discussions. We also thank Lisa Willis for secretarial work.

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