Involvement of the Paxillin Pathway in JB6 Cl41 Cell Transformation

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Abstract

Paxillin is a substrate of the Src tyrosine onco-kinase and is involved in cell transformation, cell spreading, migration, and cancer development mediated through the mitogen-activated protein kinase signaling cascades. Here, we showed that paxillin plays a key role in skin cell transformation induced by epidermal growth factor (EGF) or 12-O-tetradecanoylphorbol-13-acetate (TPA). To investigate the mechanism of paxillin’s role in cell transformation, we established a paxillin knockdown stably transfected cell line by introducing small interfering RNA-paxillin (si-paxillin). The si-paxillin cells displayed a dramatic suppression of cell proliferation and anchorage-independent cell transformation induced by EGF or TPA compared with si-mock control cells. In si-paxillin cells, decreased activator protein-1 (AP-1)–dependent luciferase activity corresponded with suppressed AP-1 DNA binding activity. Importantly, knockdown of paxillin inhibited EGF- or TPA-induced c-Jun phosphorylation at Ser63 and Ser73. Furthermore, total c-Jun protein level was dramatically decreased in si-paxillin cells and was dependent on serum deprivation time. The down-regulation of c-Jun was restored in siRNA-paxillin cells dependent on serum deprivation time. The results clearly provided evidence that paxillin regulates c-Jun stability. The phosphorylation of c-Jun at Ser63 and Ser73 was suppressed in small interfering RNA (siRNA)-paxillin cells, and the down-regulation of c-Jun was restored in siRNA-paxillin cells dependent on serum deprivation time. The down-regulation of c-Jun was restored in siRNA-paxillin cells by treatment with the proteasome inhibitor lactacystin but not by the lysosome inhibitor leupeptin. These results clearly provided evidence that paxillin regulates c-Jun protein level and plays a key role in cell transformation most likely through the regulation of c-Jun stability. (Cancer Res 2006; 66(11): 5968-74)

Introduction

Paxillin is a multidomain-containing adaptor molecule consisting of LD (LDXXLLXXL) motifs and LIM (Lin-11, Isl-1, Mec-3) domains, which provide binding sites for various proteins at focal adhesions (1–5). Paxillin has been found at the interface between the plasma membrane and the actin cytoskeleton, where it plays an important role in providing a platform for the integration and processing of adhesion and growth factor–related signals (6). Paxillin is a highly conserved protein, with 90% amino acid homology between chicken and humans (7). It is widely distributed in all tissues and cells (7, 8), suggesting that paxillin may have a unique function in cell shape change and actin cytoskeleton reorganization (9, 10).

Cell adhesion is mediated mainly through engagement of transmembrane integrin molecules by their extracellular matrix ligands, including fibronectin, vitronectin, collagen, and laminin (11–14). This interaction leads to activation, resulting in a series of intracellular responses, such as protein phosphorylation, changes in intracellular pH and calcium levels, and stimulation of mitogen-activated protein kinase (MAPK) signaling pathways (11–14). Coordination or integration of adhesion and growth factor signals is facilitated by the close physical proximity of key molecules in the signaling cascades (12). Indeed, integrins colocalize and coprecipitate with several growth factor receptors, such as the epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (12, 14). Paxillin is a scaffold protein functioning to recruit signaling proteins, including focal adhesion kinase (FAK), p210BCR/ABL, or pp60v-Src, to the plasma membrane (7, 15, 16). At the membrane, these proteins are activated by engagement of integrins with the extracellular matrix or by growth factor stimulation of receptor tyrosine kinases (RTK; ref. 6). This results in MAPK family activation, cell proliferation, and cytoskeleton rearrangement through the phosphorylation of paxillin (6). Moreover, paxillin is selectively up-regulated in certain tumor cell lines, such as HPV-18 immortalized cells and in cervical carcinoma cell lines (17), strongly suggesting that paxillin might have a role in cell transformation or tumor development. However, no report has yet implicated paxillin in these processes. The phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), and EGF are well known tumor promotion agents used to study malignant cell transformation in cell and animal models of cancer (18). The JB6 Cl41 (promotion sensitive) mouse epidermal cell line is a well-developed cell culture system for studying genetic susceptibility to transformation promotion at the molecular level (19). Moreover, we previously found that activator protein-1 (AP-1) activity and cell transformation were stimulated by TPA and EGF in the JB6 mouse epidermal cell line (20). The components of AP-1, including c-Jun, are activated by the MAPKs (21). Paxillin was first identified as a substrate for the oncogenic tyrosine kinase v-Src in v-Src-transformed cells (22). It was also shown to act as a transducer for activation signals from RTKs to downstream MAPK, such as c-Jun NH2-terminal kinases (JNK; ref. 23). Although these results have suggested an involvement of paxillin in cell transformation and tumor development, the molecular basis of signaling or related mechanism(s) are clearly not understood yet.

Here, we showed that paxillin plays a key role in skin cell transformation induced by EGF or TPA in JB6 Cl41 cells through regulation of c-Jun stability. The phosphorylation of c-Jun at Ser63 and Ser73 was suppressed in small interfering RNA (siRNA)-paxillin stably transfected cells when stimulated with EGF or TPA. In addition, total c-Jun protein level was dramatically decreased in siRNA-paxillin cells dependent on serum deprivation time. The down-regulation of c-Jun was restored in siRNA-paxillin cells by treatment with the proteasome inhibitor lactacystin but not by the lysosome inhibitor leupeptin. These results provided clear evidence showing that paxillin plays a role in regulating c-Jun protein stability and cell transformation.
GACGGTAGGTAGGGCTTTTCTT' (BbsI cohesive end underlined), and the antisense was 5'-CTAGAAAAAGAGCCCTCACCTAGGTCATCTTTTGAAATGACGGTAGGGTTTCCTT' (XbaI cohesive end underlined). Annealing, ligation, and colony screening were done as described previously (24). The oligonucleotide synthesis and sequencing of the inserted sequences in the mU6pro vector were done by Sigma. The plasmid siRNA-paxillin and pcDNA3.1 were stably transfected into JB6 Cl41 cells using the LipofectAMINE 2000 reagent. The transfected cells (si-mock or si-paxillin) were selected with G418, and paxillin expression level was confirmed by a Western blot assay using anti-paxillin (Upstate Biotechnology).

Cell proliferation assay. si-mock or si-paxillin stably transfected cells were suspended in 5% FBS/MEM at a density of 1.5 × 10^4 per mL. The cell suspension (2 mL) was placed in each well of a six-well plate and cultured in a 5% CO₂ incubator at 37°C. After incubation for various time periods, cell number was determined using a microscope.

Anchorage-independent cell transformation assay. EGF- or TPA-induced cell transformation was investigated in si-mock or si-paxillin stably transfected cells. Cells (8 × 10^3 per mL) were exposed to EGF (0.1 and 1.0 ng/mL) or TPA (10 and 20 ng/mL) in 1 mL of 0.33% basal medium Eagle agar containing 10% FBS. The cultures were incubated in a 5% CO₂ atmosphere at 37°C for 10 days (EGF) or 18 days (TPA), and the colonies were scored as described by Colburn et al. (25).

Assay of AP-1 activity. si-mock or si-paxillin stably transfected cells were transiently transfected with 2 μg of the AP-1 luciferase reporter

**Materials and Methods**

**Reagents and antibodies.** Eagle’s MEM, DMEM, and LipofectAMINE 2000 reagent were purchased from Invitrogen (Carlsbad, CA); fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Lawrenceville, GA). Restriction enzymes were purchased from New England BioLabs, Inc. (Beverly, MA). Taq DNA polymerase was from Qiagen, Inc. (Valencia, CA), and the DNA ligation kit (version 2.1) was purchased from TAKARA Bio, Inc. (Otsu, Shiga, Japan). EGF was from BD Biosciences (San Jose, CA); TPA, cycloheximide, lactacystin, and leupeptin were purchased from Sigma-Aldrich (St. Louis, MO); the luciferase assay substrate was from Promega (Madison, WI). Antibodies for Western blotting were purchased from Cell Signaling Technology, Inc. (Beverly, MA); Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); or Upstate Biotechnology, Inc. (Charlottesville, VA).

**Cell culture.** The mouse skin epidermal cell line JB6 Cl41 was cultured with MEM supplemented with 5% FBS and incubated in a 5% CO₂ atmosphere at 37°C. The human immortalized keratinocyte cell line HaCaT and the human skin epidermoid carcinoma cell line A431 were cultured with DMEM supplemented with 10% FBS incubated in a 5% CO₂ atmosphere at 37°C. The mouse embryo fibroblast cell line NIH/3T3 was cultured with DMEM supplemented with 10% FCS incubated in a 5% CO₂ atmosphere at 37°C.

**Construction of siRNA-paxillin and establishing the stable expression cell line.** To construct the siRNA-paxillin, the pU6pro vector was digested with XbaI and BbsI. The sense oligonucleotide of paxillin that was used for siRNA was 5'-TTTTGGAGCCCCTCACCTACGGTACTTCAAGAGAT- CAGCTATGATGCTGCACCTATGTTTT-3'. The antisense was 5'-CTAGAAAAAGAGCCCTCACCTAGGTCATCTTTTGAAATGACGGTAGGGTTTCCTT- ' (XbaI cohesive end underlined).

**Figure 1.** Paxillin is expressed in skin cells, and phosphorylation is induced by EGF or TPA. A, NIH/3T3 mouse embryonic fibroblast cells, JB6 Cl41 mouse skin epidermal cells, HaCaT human immortalized keratinocyte cells, or A431 human skin epidermoid carcinoma cells were cultured in 60-mm dishes and harvested when cells reached 80% confluence. Total cell lysates from the indicated cell lines were used to analyze paxillin protein levels by Western blotting using a paxillin monoclonal antibody. Equal protein loading was confirmed using a β-actin antibody on the same membrane. The protein level was normalized with β-actin protein level and quantified by comparison with NIH/3T3 cells. B, JB6 Cl41 cells were cultured, starved, and stimulated with 1 ng/mL EGF or 10 ng/mL TPA for the indicated time. Phosphorylation of paxillin (Ser118) or total paxillin protein was visualized by Western blotting using a phospho-specific antibody or an antibody against total paxillin, respectively. Equal protein loading was confirmed by stripping the membrane and reprobing with a β-actin antibody. The protein level was normalized with β-actin protein level and quantified by comparison with nontreated control.

**Figure 2.** si-paxillin inhibits cell proliferation. A, schematic diagram for the construction of the siRNA paxillin expression vector. B, DNA sequence of the two synthetic oligonucleotide primers. C, JB6 Cl41 cells were cotransfected with pcDNA3.1neo and pU6pro mock control or siRNA paxillin and selected with 400 μg/mL G418. The si-mock and si-paxillin stably transfected cells were disrupted with cell lysis buffer. Total cell lysates were used to analyze paxillin protein levels by Western blotting using a paxillin monoclonal antibody and β-actin was used to confirm equal protein loading. D, the si-mock or si-paxillin stably transfected cells were seeded (3 × 10^4 per well) in six-well plates in 5% FBS/MEM. After incubation for the indicated times, cell number was determined using a microscope. *P < 0.01, significantly higher rate of growth of si-mock stably transfected cells compared with si-paxillin stably transfected cells. Points, means of three independent experiments; bars, SD.
plasmid together with 0.1 μg of the pRL-SV40 plasmid. The cells were starved in 0.1% FBS/MEM for 24 hours and then were exposed with EGF (10 and 50 ng/mL) or TPA (5 and 20 ng/mL). The cells were disrupted with lysis buffer 12 hours later, and the luciferase activity was measured according to the manufacturer’s instructions (Promega, Madison, WI) using a luminometer (Monolight 2010, San Diego, CA). The results are expressed as relative AP-1 activity (26).

Electrophoretic mobility shift assay. AP-1 DNA binding was determined by using the electrophoretic mobility shift assay (EMSA) as described previously (27). Nuclear protein extracts were prepared from si-mock or si-paxillin stably transfected cells treated for 12 hours with EGF (5 ng/mL), TPA (10 ng/mL), UVB (4 kJ/m2), or vehicle (DMSO) as a control. An AP-1 oligonucleotide (5’-CGCTTGATGACTCAGCCGGAA-3’) was synthesized and labeled with [32P]dATP using the T4 polynucleotide kinase. Nuclear protein (3 μg) was incubated with 100,000 cpm [32P]-labeled oligonucleotide probe, 1 μg of polydeoxyinosinic-deoxycytidylic acid, and 3 μg of bovine serum albumin on ice for 10 minutes followed by incubation at room temperature for 20 minutes. To determine the binding specificity, a 5-fold excess of cold oligonucleotide was incubated in a similar reaction. The DNA-protein complexes were resolved in a 5% nondenaturing polyacrylamide gel. The gel was dried and scanned using the Storm 840 Phosphor-Image System (Molecular Dynamics, Sunnyvale, CA).

Immunoblotting. Samples containing equal amounts of protein were resolved in an 8%, 10%, or 12% SDS-polyacrylamide gel and transferred onto polyvinylidene difluoride membranes. The membranes were blocked and probed with phospho-specific antibodies against JNKs, p38, extracellular signal-regulated kinases (ERK), or c-Jun (Ser63 and Ser73; Cell Signaling Technology) or with a mouse monoclonal paxillin antibody. The Western blots were visualized using an enhanced chemiluminescence detection system (Amersham Biosciences Corp., Piscataway, NJ).

Results

Paxillin is expressed in skin epidermal cell lines. Paxillin is widely expressed in most adult human tissues with the exception of brain (8). Furthermore, paxillin is up-regulated in several types of malignant tumors (28–30). We examined paxillin expression level in mouse embryo fibroblast (NIH/3T3) cells, mouse skin epidermal cells (JB6 Cl41), human immortalized keratinocytes (HaCaT), and human skin epidermoid carcinoma cells (A431; Fig. 1A). Notably, the expression of paxillin was highest in the A431 human skin epidermoid carcinoma cells compared with the other epidermal cell lines.

Serine phosphorylation of paxillin is induced by EGF or TPA in JB6 Cl41 cells. Recently, JNK1 has been shown to directly phosphorylate paxillin (Ser178), which was implicated in the regulation of EGF-stimulated cell migration (31). We have previously shown that EGF or TPA is a well-known skin cancer promoter in vivo and in vitro, and the effects are mediated through the MAPK signaling cascades (20, 32–36). We examined the effect of EGF or TPA activation on phosphorylation of paxillin (Ser178) in the mouse skin epidermal JB6 Cl41 cell line. EGF treatment induced strong phosphorylation of paxillin (Ser178) at 15 minutes, and the phosphorylation was maintained for at least 3 hours (Fig. 1B). On the other hand, TPA induced phosphorylation of paxillin (Ser178) at 15 minutes, and the phosphorylation increased gradually up to 12 hours (Fig. 1B). These results indicated that phosphorylation of paxillin (Ser178) was induced by treatment with tumor promoters, such as EGF or TPA.
siRNA knockdown of paxillin inhibits cell proliferation. To further delineate the role of paxillin in cell proliferation and transformation, an siRNA against paxillin (si-paxillin) was constructed by replacing the green fluorescence protein region of the pU6pro vector with si-paxillin primers (Fig. 2A and B). We confirmed the efficiency of siRNA in knocking down paxillin protein level in stably transfected cells by Western blotting. Paxillin protein level was dramatically decreased in si-paxillin stably transfected cells compared with si-mock stably transfected cells (Fig. 2C). Furthermore, we found that si-paxillin stably transfected cells delayed the rate of spreading agreeing with others who observed similar results in paxillin-deficient cells (data not shown; ref. 37). To examine the effect of paxillin on cell proliferation, we analyzed a cell growth curve for si-mock and si-paxillin stably transfected cells. The results indicated that cell proliferation of si-paxillin stably transfected cells was significantly suppressed compared with si-mock stably transfected cells (*, P < 0.01; Fig. 2D). These results suggested that paxillin is involved in cell proliferation.

siRNA knockdown of paxillin inhibits anchorage-independent tumor promoter-induced cell transformation. The mouse skin epidermal JB6 Cl41 cell system is a well-developed model for studying tumor promotion under anchorage-independent growth conditions (20). This model was, therefore, used to determine the role of paxillin in neoplastic cell transformation. si-mock or si-paxillin stably transfected cells were stimulated with EGF (0, 0.1, and 1.0 ng/mL) or TPA (0, 10, and 20 ng/mL) as described in Materials and Methods. The number of colonies formed after treatment with EGF (Fig. 3A and B) or TPA (Fig. 3C and D) was significantly (*, P < 0.01) decreased in the si-paxillin stably transfected cells compared with the si-mock stably transfected cells (*, P < 0.01). Furthermore, phosphorylation of ERKs, p38, or JNKs in si-mock or si-paxillin stably transfected cells delayed the rate of spreading agreeing with others who observed similar results in paxillin-deficient cells (data not shown; ref. 37).

Figure 4. si-paxillin inhibits EGF- or TPA-induced AP-1 activity and DNA binding activity. AP-1 transactivation has been shown to be required for neoplastic transformation in JB6 Cl41 cells (20). To test whether the inhibition of cell transformation by si-paxillin involves suppression of AP-1 activity, we transfected an AP-1 linked luciferase reporter plasmid into si-mock or si-paxillin stably transfected cells and stimulated the cells with EGF or TPA. The results indicated that AP-1 activation was induced by EGF or TPA in si-mock stably transfected cells but not in si-paxillin stably transfected cells (Fig. 4A and B). To further study the molecular basis of paxillin’s inhibitory effect on AP-1 activation, AP-1 DNA-binding activity was analyzed by EMSA. Our results indicated that EGF, TPA, or UVB-induced AP-1 DNA binding activity was decreased in si-paxillin stably transfected cells compared with si-mock stably transfected cells (Fig. 4C). Taken together, these results suggested that AP-1 activation might be mediated through the paxillin pathway.

si-paxillin inhibits EGF- or TPA-induced phosphorylation of JNKs and c-Jun. MAPKs contribute to the activation of AP-1 in response to a broad range of extracellular stimuli (38, 39). To test whether the paxillin pathway is associated with activation of the MAPKs, we used Western blotting to detect EGF- or TPA-induced phosphorylation of ERKs, p38, or JNKs in si-mock or si-paxillin stably transfected cells. Our results indicated that EGF- or TPA-induced phosphorylation of JNKs was suppressed in si-paxillin stably transfected cells (Fig. 5A and B). Furthermore, phosphorylation of ERKs, p38, or JNKs was not altered in si-paxillin stably transfected cells compared with si-mock stably transfected cells. These results indicated that EGF or TPA induced phosphorylation of ERKs, p38, or JNKs was not altered in si-paxillin stably transfected cells compared with si-mock stably transfected cells. Therefore, the inhibition of cell transformation by si-paxillin is not due to the inhibition of phosphorylation of ERKs, p38, or JNKs.

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of c-Jun and total c-Jun expression were also decreased in si-paxillin stably transfected cells (Fig. 5A and B). On the other hand, our results indicated that EGF- or TPA-induced phosphorylation of ERKs and p38 were similar in both si-mock and si-paxillin stably transfected cells (data not shown). These results strongly suggested that paxillin is required for EGF- or TPA-induced cell transformation mediated through the activation and phosphorylation of JNKs and c-Jun.

si-paxillin is involved in c-jun protein down-regulation. The c-Jun protein is degraded through the ubiquitin pathway, which is also responsible for regulating the stability of other short-lived proteins, such as c-Fos, c-Myc, and p53 (40, 41). Phosphorylation of c-Jun by JNKs can protect c-Jun from ubiquitination (42). Our results indicated that the knockdown of paxillin caused a dramatic decrease in c-Jun protein expression without the addition of EGF or TPA (Fig. 5A and B). We also observed that TPA-, EGF-, or UVB-induced c-Jun protein expression levels were decreased in si-paxillin stably transfected cells (Fig. 6A), which further agrees with the observation of decreased AP-1 activation and DNA-binding activity. This result suggested that paxillin also might play a role in stabilizing c-Jun protein expression, which is independent of EGF- or TPA-induced signal transduction. To examine whether paxillin is essential for c-Jun protein stability, si-mock or si-paxillin stably transfected cells were treated for 6 hours under serum-deprived conditions, with cycloheximide, an inhibitor of protein synthesis (10 μg/mL), and either lactacystin (10 μmol/L), an inhibitor of the proteasome degradation pathway, or leupeptin (100 μg/mL), an inhibitor of the lysosomal degradation pathway. Our results indicated that expression of the c-Jun protein in si-paxillin stably transfected cells was at least partially restored by treatment with the proteasome inhibitor lactacystin but not by the lysosome inhibitor leupeptin (Fig. 6C). β-Actin protein levels were not affected under these conditions. Overall, these results indicate that paxillin is required for cell transformation and AP-1 transactivation mediated through the JNK pathway.

Discussion

Paxillin has been classified as a molecular adaptor or scaffold protein because it contains many protein binding modules, such as LIM domains and LD motifs. These domains and motifs are important for binding with signaling molecules, such as FAK, vinculin, COOH-terminal Src kinase, Src, and papilloma virus E6 oncogene (15, 43–47), and serve as phosphorylation targets of intracellular protein kinases (15, 31, 43, 48). Importantly, these

Figure 5. si-paxillin inhibits EGF- or TPA-induced phosphorylation and protein level of c-Jun. si-mock or si-paxillin stably transfected cells (80% confluence) were starved by replacing the medium with 0.1% FBS/MEM and culturing for 24 hours. Cells were then treated or not treated with 1.0 ng/mL EGF (A) or 10 ng/mL TPA (B) for 15 minutes. After treatment, the cells were harvested and subjected to Western blotting to detect phosphorylation of various MAPKs and phosphorylation and protein levels of c-Jun. Equal protein loading was confirmed by stripping the membrane and reprobing with a β-actin antibody.

Figure 6. si-paxillin is involved in c-Jun protein down-regulation. A, si-mock or si-paxillin stably transfected cells (80% confluence) were starved by replacing the medium with 0.1% FBS/MEM and culturing for 24 hours. Cells were then treated or not treated with 5 ng/mL EGF, 10 ng/mL TPA, or 4 J/m² UVB and harvested after 12 hours. Nuclear proteins from the preparations above were used to analyze EGF-, TPA-, or UVB-induced c-Jun protein levels by Western blotting. B, si-mock or si-paxillin stably transfected cells (80% confluence) were starved by replacing the medium with 0.1% FBS/MEM and culturing for the indicated time. The cells were harvested and subjected to Western blotting to detect c-Jun protein levels. C, si-mock or si-paxillin stably transfected cells (80% confluence) were treated with cycloheximide (10 μg/mL), an inhibitor of protein synthesis, and lactacystin (10 μmol/L), an inhibitor of the proteasome degradation pathway, or leupeptin (100 μg/mL), an inhibitor of the lysosomal degradation pathway for 6 hours under serum-deprived conditions.
Our results also showed that paxillin is phosphorylated at Ser178 following EGF stimulation (31). Our experiments also showed that paxillin is phosphorylated at Ser178 following stimulation by EGF or TPA. Therefore, we hypothesized that paxillin may have functions in addition to cell migration. In our experiments, we showed that knockdown of paxillin by siRNA resulted in decreased proliferation, a delayed rate of cell spreading, and a substantial decrease in EGF- or TPA-induced neoplastic cell transformation. These changes were associated with a suppression of EGF- or TPA-induced AP-1 activation and DNA binding and a down-regulation of JNKs and c-Jun phosphorylation and c-Jun protein expression levels. These results clearly indicated that paxillin is involved in mediating cell proliferation and transformation through the regulation of the protein stability of the JNKs and c-Jun proteins.

There are two possibilities for the observed increase in paxillin protein level with stimulation by EGF or TPA. EGF and TPA stimulate MAPK signaling cascades and activate downstream target transcription factors, resulting in increased target gene expression. One of the most well known downstream targets is the AP-1 transcription factor complex, which most commonly exists as a dimer consisting of c-Jun and c-Fos proteins. In reverse transcription-PCR analysis, we found that EGF and TPA increased paxillin mRNA level in a time-dependent manner (data not shown). Although paxillin can be phosphorylated by JNK1 and is involved in cell migration (31), we found that knockdown of paxillin decreased the phosphorylation level of JNKs (Fig. 5A and B). This result suggested that paxillin can affect the upstream signaling pathway of JNKs. Overexpression of wild-type FAK induces phosphorylation of p130Cas and paxillin; however, it did not activate ERKs and JNKs (51). The COOH-terminal truncated form of the myr-FAK has an ability to associate with paxillin-associated complexes in vivo and activates JNKs. Conversely, overexpression of COOH-terminal domain of myr-FAK fails to associate with paxillin or activate JNKs, suggesting that the NH2-terminal of FAK is involved in paxillin binding, and results in JNKs activation (52). These FAK/paxillin/JNK signaling pathways may be mediated through protein kinase PAK, which is an upstream component of the JNK pathway (53, 54), or protein kinase C (55).

To date, although a function of paxillin in cell transformation and tumor development has been suggested, no direct evidence or specific molecular mechanism has yet been published. In the present study, the function of paxillin as an adaptor or "protein stabilizer" is a novel mechanism for understanding how proteins can be protected from the ubiquitination-dependent degradation pathway during the cell transformation process. Therefore, paxillin may be a good target molecule for new chemopreventive and therapeutic drugs.

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