Focal Adhesion Kinase Is Negatively Regulated by Phosphorylation at Tyrosine 407*

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Focal adhesion kinase (FAK) mediates signal transduction in response to multiple extracellular inputs via tyrosine phosphorylation at specific residues. Although several tyrosine phosphorylation events have been linked to FAK activation and downstream signal transduction, the function of FAK phosphorylation at Tyr407 was previously unknown. Here, we show for the first time that phosphorylation of FAK Tyr407 increases during serum starvation, contact inhibition, and cell cycle arrest, all conditions under which activating FAK Tyr397 phosphorylation decreases. Transfection of NIH3T3 cells with a phosphorylation-mimicking FAK 407E mutant decreased autophosphorylation at Tyr397 and inhibited both FAK kinase activity in vitro and FAK-mediated functions such as cell adhesion, spreading, proliferation, and migration. The opposite effects were observed in cells transfected with nonphosphorylatable mutant FAK 407F. Taken together, these data suggest the novel concept that FAK Tyr407 phosphorylation negatively regulates the enzymatic and biological activities of FAK.

Focal adhesion kinase (FAK)3 is a nonreceptor cytoplasmic tyrosine kinase that modulates various cell functions, including survival, proliferation, and migration (1, 2). Occupying an important receptor-proximal position in the signaling cascade, FAK mediates signal transduction in response to adhesion and/or binding of growth factors. The ability of FAK to transduce downstream signal(s) is dependent on its phosphorylation at tyrosine residues (3). Activation and phosphorylation of FAK leads to recruitment of a number of SH2 and SH3 domain-containing proteins that mediate signaling via multiple downstream pathways. These include the Src family kinases (4), phosphatidylinositol 3-kinase (PI3K), p130Cas (5, 6), and Grb2 (7). Six tyrosine phosphoacceptor sites have been identified in FAK, Tyr397, Tyr407, Tyr576, Tyr577, Tyr861, and Tyr925, most of which appear to play positive regulatory roles. As an example of FAK signaling, when integrin is activated, FAK is recruited to focal contacts and autophosphorylated at Tyr397 (8). This creates a high-affinity binding site for the SH2 domain of Src family tyrosine kinases and the p85 subunit of phosphoinositide 3-kinase, allowing their recruitment and activation. The recruitment of Src leads to additional phosphorylation on Tyr576, Tyr577, and Tyr861. Phosphorylation at Tyr576 and Tyr577 further activates FAK kinase activity (9), whereas at Tyr861 creates a binding site for the protein complex containing the adaptor Grb2 and the ras guanosine 5′-triphosphate exchange factor mSos, which in turn activates the mitogen-activated protein kinase pathway. Phosphorylation at Tyr861 is less well understood but has been detected in several cell lines, including prostate carcinoma cells (10), metastatic breast cancer cells (11), vesicular endothelial cells (12), and Ras-transformed fibroblasts (13). Leu and Maa (14) suggested that phosphorylation at Tyr861 might enhance Tyr397 phosphotyrosine phosphorylation of Tyr397, and our group recently reported that Tyr861 phosphorylation was crucial for H-ras-induced transformation (15), implying that Tyr861 phosphorylation is likely to potentiate the functions of FAK.

In contrast to the other phosphotyrosine residues, phosphorylation at Tyr407 has not been extensively studied. Src has been proposed to regulate phosphorylation of FAK Tyr407, because phosphorylation of Tyr407 was significantly and adhesion-dependently elevated in the presence of c-Src (16). However, Src kinase-independent phosphorylation at Tyr407 has been also proposed, based on increased tyrosine phosphorylation of FAK Tyr407 in a colon cancer cell line (KM12C) expressing kinase-deficient Src proteins (17).

Furthermore, although it is well known that other tyrosine phosphorylation events are linked to FAK activation and downstream signal transduction, the function of FAK phosphorylation at Tyr407 remains unknown. A recent study showed that exposure of endothelial cells to vascular endothelial growth factor-induced Tyr407 phosphorylation, suggesting that Tyr407 phosphorylation may play a role in transducing the vascular endothelial growth factor signals, which trigger assembly of focal adhesions and endothelial cell migration (18). Therefore, it is highly possible that phosphorylation of FAK at Tyr407 plays one or more roles in FAK functions. Here we show for the first time that Tyr407 phosphorylation negatively regulates the enzymatic and biological activities of FAK.

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§ The abbreviations used are: FAK, focal adhesion kinase; SFM, serum-free medium; GST, glutathione S-transferase; HA, hemagglutinin; PI3K, phosphatidylinositol 3-kinase; FBS, fetal bovine serum; CO, cholesterol; BrdU, bromodeoxyuridine; MITT, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide; PBS, phosphate-buffered saline; ERK, extracellular signal-regulated kinase; siRNA, small interfering RNA; mFAK, mouse FAK; cFAK, chicken FAK.
time that FAK Tyr\textsuperscript{407} phosphorylation appears to function in the negative regulation of FAK activity and function.

**MATERIALS AND METHODS**

**Cell Culture**—NIH3T3 cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 10 units/ml penicillin, and 10 \( \mu \text{g/ml} \) streptomycin. NIH3T3 cells stably expressing HA-tagged FAK Y407F were maintained in Dulbecco’s modified Eagle’s medium with 10% FBS, 250 \( \mu \text{g/ml} \) G418, 10 units/ml penicillin, and 10 \( \mu \text{g/ml} \) streptomycin.

**Reagents and Antibodies**—Chloroquine (CQ), deferoxamine, trichostatin A, propidium iodide, bromodeoxyuridine (BrdUrd), and poly(Glu-Tyr) were purchased from Sigma, and monoclonal antibody against HA (12CA5) was purchased from Roche Applied Science. Polyclonal antibodies against p21 and p27, and monoclonal antibodies against ERK2 (K-23), phospho-specific ERK (E-4), and PI3K were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-Tyr(P) antibody (4G10) and the anti-Src antibody (GD11) were obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). The monoclonal antibody against FAK and polyclonal antibodies to a phosphorylation site specific against FAK[Tyr(P)\textsuperscript{397}] and FAK[Tyr(P)\textsuperscript{407}] were purchased from BioSource Quality Controlled Biochemicals, Inc. (Morgan Hill, CA).

**Construction of Mutant FAK Mammalian Expression Vectors**—The full-length cDNA encoding FAK was subjected to site-directed mutagenesis using the Transformer site-directed mutagenesis kit (Clontech, Palo Alto, CA). The synthetic oligonucleotides GGA AGA CAC ATT TAC CAT GCC CTC G (Y\textsuperscript{407} \rightarrow \text{Phe}) and GGA AGA CAC AGA AAC CAT GCC CTC G (Y\textsuperscript{407} \rightarrow \text{Glu}) were used to change Tyr\textsuperscript{407} to phenylalanine or glutamic acid. The cDNAs encoding wild-type FAK and mutant Y407F were inserted into pRC/cyto-megaLLV5 (Amersham Biosciences). The PCR products were cloned into the BamHI/EcoRI site of pGEX-4T-1 expression vector (Amersham Biosciences).

**Transfection**—Transient transfections were carried out using Lipofectamine reagent (Invitrogen) as described by the manufacturer. In brief, NIH3T3 cells were plated in 60-mm dishes and grown to \( \sim 80\% \) confluence for 24 h. The cells were then transfected with a mixture of 15 \( \mu \text{l} \) of Lipofectamine and 4 \( \mu \text{g} \) of plasmid DNA. After 5 h, the transfection mixture was removed and replaced with medium containing 10% FBS. For stable transfections, transfected cell populations were selected by the continuous presence of 250 \( \mu \text{g/ml} \) of G418.

**Synthesis and Transfection of siRNA Constructs**—To design oligonucleotides targeting the mouse FAK mRNA for degradation, siRNA Design of Ambion was used. The chosen targeted FAK siRNA sequence (5' - AATGCTCTAGAGAAAGTCC-3') was chemically synthesized by Ambion (Austin, TX), and the negative control siRNAs were purchased from Ambion. In vitro cotransfections were performed with 500 \( \text{pm} \) of mouse siRNAs and 4 \( \mu \text{g} \) of constructs encoding chicken FAK with and without introduced mutations (Y407Y, Y407F, and Y407E), using Lipofectamine 2000 (Invitrogen), according to the manufacturer’s protocols.

**Cell Proliferation Assay**—Cell proliferation was measured by a colorimetric assay using MTT. In brief, NIH3T3 cells transfected with each of the generated plasmids were harvested with 0.05% trypsin/EDTA and seeded into 35-mm dishes at \( 1 \times 10^5 \) cells/dish. After the cells were allowed to attach, the medium containing 0.5 mg/ml MTT was added to each plate in a volume of 100 \( \mu \text{l} \), and the cells were incubated for 1 h. The medium was then removed, 200 \( \mu \text{l} \) of dimethyl sulfoxide was added, and the plates were incubated for half an hour at room temperature. The mean concentration of absorbance at 570 nm in each set of all samples was measured using a 96-well microtiter plate reader (Dyntech, Chantilly, VA).

**BrdUrd Incorporation Assay**—The cells were pulsed with 10 \( \mu \text{M} \) BrdUrd for 2 h, harvested, fixed in 70% ethanol at 4 °C for 30 min, and resuspended in 2 \( \times \) 10\textsuperscript{5} HCl. The reactions were neutralized with 0.1 M sodium tetraborate for 2 min, the cells were stained with fluorescein isothiocyanate-conjugated anti-BrdUrd antibody in 0.5 ml of 1% bovine serum albumin and 0.5% Tween 20 in phosphate-buffered saline (PBS) for 1 h at room temperature in the dark. The cells were then washed once and incubated in 0.5 ml of PBS containing 5 \( \mu \text{g/ml} \) propidium iodide for 30 min prior to analysis with a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA) and the CellQuest\textsuperscript{TM} software.

**Cell Adhesion Assays**—Fibronectin was diluted to 10 \( \mu \text{g/ml} \) in serum-free medium (SFM) and dispensed to 12-well plates (10 \( \mu \text{g/ml} \) that were incubated at room temperature for at least 1 h to allow adsorption. The plates were then washed with PBS, blocked with 0.2% heat-inactivated bovine serum albumin for 1 h, and then washed with SFM (2 \( \times \) 10 min). Cultured cells were detached from culture plates with 0.05% trypsin and 0.53 mM EDTA, suspended in SFM containing 0.25 mg/ml of soybean trypsin inhibitor, and centrifuged. The cells were then resuspended in SFM, plated onto fibronectin-coated plates, and incubated for the indicated durations at 37 °C. For analysis of cell morphology, the cells were visualized with an inverted microscope (Zeiss) at 20× magnification.

**Migration Assay**—Fibronectin (10 \( \mu \text{g/ml} \) was added to each well of a 24-well Transwell plate (8-\( \mu \text{m} \) pore size; Costar), and the membranes were allowed to dry for 1 h at 25 °C. Cells transfected with the various constructs (5 \( \times \) \( 10^5 \) cells) were added to the upper compartment of each well, and the plate was incubated for 6 h at 37 °C in a 5% \( \text{CO}_2 \) atmosphere. Nonmigrated cells on the upper membrane were removed with a cotton swab. Migrated cells (located on the lower surface of the filters) were fixed for 5 min in methanol, stained with 0.6% hematoxylin and 0.5% eosin, and then counted.

**Subcellular Fractionation**—After washing twice with PBS (500 \( \mu \text{l} / 10-\text{cm diameter plate}) , 150 \( \mu \text{l} \) of hypotonic solution (20 mM Tris/HCl, pH 7.5, 2 mM \( \beta \)-mercaptoethanol, 5 mM EGTA, 2
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mm EDTA) containing a protease inhibitor mixture (1 μg/ml aprotinin, 1 μg/ml antipain, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, 20 μg/ml phenylmethylsulfonyl fluoride) were applied to the culture plates. The cells were subsequently scraped off the plates and homogenized on ice. The homogenate was centrifuged at 13,000 × g for 15 min at 4 °C. The resulting supernatant represented the cytosolic fraction. The membrane fraction was collected by solubilizing the remaining pellet in radioimmunoprecipitation assay buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 2 mM Na3VO4) containing a protease inhibitor mixture.

**Immunoblotting**—The cells were washed twice with PBS and were lysed in radioimmunoprecipitation assay buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 2 mM Na3VO4) containing a protease inhibitor mixture (1 μg/ml aprotinin, 1 μg/ml antipain, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, 20 μg/ml phenylmethylsulfonyl fluoride). The lysates were clarified by centrifugation at 14,000 rpm for 15 min at 4 °C, denatured with SDS sample buffer, boiled, and analyzed by SDS-PAGE. The proteins were transferred onto polyvinylidene difluoride membranes (Amersham Biosciences), incubated with the appropriate primary antibodies, detected with species-specific horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences), and visualized by enhanced chemiluminescence (ECL; Amersham Biosciences). The membranes were then reprobed with anti-ERK2 antibody. The results shown represent the averages of three independent experiments.

**RESULTS**

**FAK Tyr<sup>407</sup> Phosphorylation Appears to Be Inversely Correlated with Cell Proliferation**—To investigate the role of phosphorylation at FAK residue Tyr<sup>407</sup>, we examined this site-specific phosphorylation under various cell conditions (Fig. 1). FAK Tyr<sup>407</sup> phosphorylation was found to be higher in NIH3T3 cells treated with the cell cycle arresting agents, CQ and deferoxamine (Fig. 1A), as well as in serum-starved cells (Fig. 1B) and cells grown to achieve higher density saturation at confluence versus exponentially growing cells (Fig. 1C). Because both cell proliferation and FAK activity are decreased under conditions of serum starvation, contact inhibition, and cell cycle arrest, these results seem to suggest that FAK Tyr<sup>407</sup> phosphorylation might decrease the ability of FAK to regulate cell proliferation. Consistent with this notion, we found that FAK Tyr<sup>407</sup> phosphorylation was inversely related with activation-associated phosphorylation at FAK Tyr<sup>397</sup> (Fig. 1).

To further investigate the potential negative role of FAK Tyr<sup>407</sup> phosphorylation in cell proliferation, we used site-directed mutagenesis to replace Tyr<sup>407</sup> with a nonphosphorylatable phenylalanine residue (407F) and transfected vectors encoding HA-tagged 407F into NIH3T3 cells (Fig. 2). Compared with vector-transfected cells, those expressing wild type (FAK) and FRNK showed similar cell numbers. In contrast, for 72 h mutant FAK 407F-transfected cells showed slight but sig-
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FAK Tyr407 Phosphorylation Negatively Regulates Autophosphorylation and Kinase Activity—Our finding that the phosphorylation levels of Tyr407 and Tyr397 were inversely correlated promptly us to investigate whether FAK Tyr407 phosphorylation might negatively regulate FAK activity. NIH3T3 cells were transfected with the empty vector, wild-type FAK (407Y), nonphosphorylatable mutant FAK (407F), or phosphorylation-mimicking mutant FAK (407E). Interestingly, both total tyrosine phosphorylation of FAK and autophosphorylation at Tyr397 was increased in cells transfected with FAK 407F and decreased in cells transfected with FAK 407E, compared with cells expressing wild-type FAK (Fig. 4A, top panel). Similarly, in vitro kinase assay showed that FAK kinase activity was increased in cells expressing FAK 407F and decreased in cells expressing FAK 407E, compared with cells expressing wild-type FAK, FAK 407Y (Fig. 4A, bottom panel). Phosphorylation of FAK Tyr397 creates a high affinity binding site for PI3K and Src family kinases. Consistent to the altered levels of Tyr397 phosphorylation, interactions of FAK with PI3K and Src were decreased in cells transfected with FAK 407E mutant and increased in cells transfected with FAK 407F mutant versus those expressing wild-type FAK (Fig. 4B). These results collectively suggest that FAK Tyr407 phosphorylation may inhibit FAK Tyr397 phosphorylation.

Because Tyr397 and Tyr407 are located close to one another in the linker region of the FAK protein, we examined whether phosphorylation of one affects that of the other. We generated
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A recombinant GST-FAK 407 polypeptide containing phosphorylatable Tyr\(^{397}\) and wild-type Tyr\(^{407}\) (407Y) or mutants containing either Glu (407E) or Phe (407F). FAK immunocomplexes containing these peptides were used for \textit{in vitro} kinase assays. Interestingly, Tyr\(^{397}\) phosphorylation was 2-fold higher in experiments containing GST-FAK 407F and decreased in those containing GST-FAK 407E, compared with assays involving GST-FAK 407Y (Fig. 4C). These findings seem to indicate that FAK Tyr\(^{407}\) phosphorylation might inhibit the access of additional phosphates on Tyr\(^{397}\), perhaps because of increased negative charge repulsion. It has been known that FAK molecules phosphorylated at Tyr\(^{397}\) reside close to the plasma membrane (21, 22), and the absence of Tyr\(^{397}\) phosphorylation decreases its residency at focal adhesions but not in cytosol (22). Consistent with this notion, higher levels of Tyr\(^{407}\) phosphorylation were observed in cytosolic FAK versus membrane-bound FAK (Fig. 4D). Collectively, these data suggest that FAK Tyr\(^{407}\) phosphorylation negatively regulates autophosphorylation at Tyr\(^{397}\) and FAK kinase activity.

**FAK Tyr\(^{407}\) Phosphorylation Negatively Regulates FAK Functions**—Because FAK plays a critical role in integrin-mediated signal transduction, where it acts as a cytosolic kinase to phosphorylating cytoskeletal proteins, and because integrin-mediated signal transduction requires Tyr\(^{397}\) phosphorylation (23), we speculated that FAK Tyr\(^{407}\) phosphorylation might negatively regulate FAK function. To examine this possibility, we designed a unique 21-bp small interfering RNA (siRNA) sequence targeted against the mouse FAK (mFAK) mRNA and used this siRNA to knock down mFAK expression in NIH3T3 cells (Fig. 5A). Expectedly, NIH3T3 cells transfected with mFAK siRNA showed decreased expression of FAK (Fig. 5A, compare \textit{first} and \textit{second lanes}) as well as decreased cell adhesion and migration on fibronectin (data not shown, but refer to Figs. 6A and 7). To further investigate the role of phosphorylation at FAK residue Tyr\(^{407}\), we then re-expressed recombinant HA-tagged chicken FAK (cFAK) in the knockdown NIH3T3 cells and compared the knockdown cells with those expressing recombinant wild-type or mutant FAK proteins. Consistent with the results reported above, FAK Tyr\(^{397}\) phosphorylation was increased in knockdown cells transfected with cFAK 407F and decreased in those expressing cFAK 407E, as compared with the knockdown cells transfected with cFAK 407Y (data not shown but refer to Fig. 6B). Similarly, proliferation and BrdUrd incorporation were increased in knockdown cells expressing cFAK 407F and decreased in those expressing cFAK 407E, as compared with the knockdown cells transfected with cFAK 407Y (Fig. 5, \textit{B} and \textit{C}). We then examined integrin-mediated cell adhesion in FAK knockdown cells and those re-expressing mutant or wild-type cFAK. The cells were detached and replated on fibronectin and observed for spreading. By 60 min about 38\% of the mFAK knockdown NIH3T3 cells showed cell spreading on fibronectin. In contrast, about 82\% of cells re-expressing wild-type cFAK completed cell spreading. Interestingly, re-expression of cFAK 407F further enhanced adhesion and spreading, whereas re-expression of cFAK 407E decreased adhesion and spreading, as compared...
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Although phosphorylation of FAK at Tyr407 was previously reported (16, 17), the function of this event was virtually unknown until this point. Here, we provided the first evidence that FAK Tyr407 phosphorylation has a negative regulatory function. Unlike the other five phosphorylatable tyrosine residues, which showed increased phosphorylation upon activation of FAK, higher levels of FAK Tyr407 phosphorylation were observed under conditions of basal FAK activity and relatively low levels of FAK Tyr397 phosphorylation. Both Tyr397 autophosphorylation and FAK kinase activity were decreased in cells transfected with the phosphorylation-mimicking FAK 407E mutant and increased in cells transfected with the nonphosphorylatable FAK 407F mutant (Fig. 4). In addition, FAK functions such as cell adhesion, spreading, migration, and proliferation were decreased in cells transfected with FAK 407E and increased in cells transfected with FAK 407F, as compared with cells transfected with FAK Tyr407 phosphorylation is not mediated by Src family

Phosphorylation of Focal Adhesion Kinase during Cell Cycle Arrest—Because Src has been proposed to regulate phosphorylation of FAK Tyr407 (16), we investigated whether Src family kinase plays a role in FAK Tyr407 phosphorylation during cell cycle arrest. NIH3T3 cells lysates were incubated with [γ-32P]ATP and GST-FAK407 as a substrate to allow phosphorylation, and phosphorylated proteins were analyzed by autoradiography (Fig. 8). Interestingly, GST-FAK407 phosphorylation was observed only in CQ-treated cell lysate (Fig. 8A, left panels), and it was also seen in the presence of PP1, the Src family kinase inhibitors (Fig. 8A, right panels). In addition, consistent to the previous report (13), trichostatin A caused an increase of FAK Tyr407 phosphorylation in H-ras-transformed NIH3T3 cells even in the presence of PP1. Therefore, it seems that FAK Tyr407 phosphorylation is not mediated by Src family kinase during cell cycle arrest.

**DISCUSSION**

Although phosphorylation of FAK at Tyr407 was previously reported (16, 17), the function of this event was virtually unknown until this point. Here, we provided the first evidence that FAK Tyr407 phosphorylation has a negative regulatory function. Unlike the other five phosphorylatable tyrosine residues, which showed increased phosphorylation upon activation of FAK, higher levels of FAK Tyr407 phosphorylation were observed under conditions of basal FAK activity and relatively low levels of FAK Tyr397 phosphorylation. Both Tyr397 autophosphorylation and FAK kinase activity were decreased in cells transfected with the phosphorylation-mimicking FAK 407E mutant and increased in cells transfected with the nonphosphorylatable FAK 407F mutant (Fig. 4). In addition, FAK functions such as cell adhesion, spreading, migration, and proliferation were decreased in cells transfected with FAK 407E and increased in cells transfected with FAK 407F, as compared with cells transfected with FAK Tyr407 phosphorylation is not mediated by Src family kinase.
domains of integrins activate FAK by relieving FERM-mediated autoinhibition. Here, we provide the first evidence supporting a Tyr407 phosphorylation-mediated negative regulatory mechanism for FAK. Specifically, we show that FAK-407 phosphorylation negatively regulates important FAK functions including cell adhesion, proliferation, and migration in NIH3T3 cells (Figs. 6 and 7). This negative regulation could exist in addition to FERM-mediated inhibition, or it could be a downstream event initiated by FERM.

Our experiments further revealed that FAK Tyr407 phosphorylation appears to inhibit the activating phosphorylation of Tyr397. This may be mediated by physical hindrance of the tyrosine binding-like domain. Future work will be required to determine the precise mechanism(s) by which inactivating phosphorylations between FERM and kinase domains. This hypothesis is consistent with the observation that FERM contains a phospho-tyrosine mimicking GST-FAK 407E (Fig. 4).

Another possibility is that FAK Tyr407 phosphorylation may be involved in FERM-mediated autoinhibition of FAK. If the FERM domain represses the catalytic activity of the enzyme by intramolecular autoinhibition, the linker domain of FAK, which contains Tyr407, might participate by stabilizing interactions between FERM and kinase domains. This hypothesis is supported by the observation that FERM contains a phosphotyrosine binding-like domain. Future work will be required to determine the precise mechanism(s) by which inactivating phosphorylation at Tyr407 inhibits activating phosphorylation at Tyr397. In addition, Src has been proposed to regulate FAK phosphorylation at Tyr407 (16). We found, however, that the recombinant FAK polypeptide containing Tyr407 was not phosphorylated by Src family kinase but phosphorylated by cell lysate from serum-starved NIH3T3 cells (Fig. 8), implying negative regulation through FAK Tyr407 phosphorylation by another cytosolic tyrosine kinase.

In sum, we herein show for the first time that FAK Tyr407 phosphorylation contributes to negative regulation of kinase activity and decreased autophosphorylation at Tyr397, leading to negative regulation of FAK-related functions in adhesion-mediated signal transduction. We are currently attempting to identify tyrosine kinase and tyrosine phosphatase that respectively phosphorylate and dephosphorylate FAK Tyr407, in an effort to clarify the precise negative regulatory mechanism(s) of FAK Tyr407 phosphorylation. However, the present work provides the first evidence for negative regulation of FAK activity and function by phosphorylation at Tyr407.

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