Focal adhesion kinase N-terminus in breast carcinoma cells induces rounding, detachment and apoptosis

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Focal adhesion kinase (FAK) has a central role in adhesion-mediated cell signalling. The N-terminus of FAK is thought to function as a docking site for a number of proteins, including the Src-family tyrosine kinases. In the present study, we disrupted FAK signalling by expressing the N-terminal domain of FAK (FAK-NT) in human breast carcinoma cells, BT474 and MCF-7 lines, and non-malignant epithelial cells, MCF-10A line. Expression of FAK-NT led to rounding, detachment and apoptosis in human breast cancer cells. Apoptosis was accompanied by dephosphorylation of FAK Tyr[397], degradation of the endogenous FAK protein and activation of caspase-3. Over-expression of FAK rescued FAK-NT-mediated cellular rounding. Expression of FAK-NT in non-malignant breast epithelial cells did not lead to rounding, loss of FAK phosphorylation or apoptosis. Thus FAK-NT contributes to cellular adhesion and survival pathways in breast cancer cells which are not required for survival in non-malignant breast epithelial cells.

Key words: apoptosis, breast cancer cell, caspase activation, protein degradation.

INTRODUCTION

Focal adhesion kinase (FAK) is a 125 kDa protein tyrosine kinase localized to focal adhesions [1,2], which are sites of cellular interactions with the extracellular matrix. FAK was first identified in v-*src*-transformed fibroblasts as a tyrosine phosphorylated protein [3] that co-precipitated with v-Src [4]. Tyrosine phosphorylation of FAK occurs in response to clustering of integrins [5], during formation of focal adhesions and cell spreading [1,6,7]. In addition, FAK can be tyrosine phosphorylated by other stimuli, such as bombesin [8], platelet-derived growth factor [9] and hepatocyte growth factor [10,11]. FAK associates with a number of key signalling proteins, including phosphoinositide 3-kinase [12], p130^{CAS} [13,14] and growth-factor-receptor-bound protein (Grb)2 [15,16], suggesting that it may function as a switchable adaptor protein, recruiting or anchoring proteins to focal adhesions.

We previously identified the human homologue of FAK in a PCR-based screen for protein kinases expressed in high-grade sarcomas [17]. FAK is over-expressed in breast, colon and thyroid tumours, as well as sarcomas, and FAK is amplified in human tumours [19]. FAK plays an essential role in tumour cell adhesion [18], as well as survival, because interruption of FAK function by antisense oligonucleotides led to irreversible loss of adhesion and viability [20]. FAK has been proposed to be part of a survival signalling pathway in normal cells [21–23] and has an association with cell motility [24,25].

The FAK protein consists of a highly conserved catalytic domain flanked by large N- and C-terminal domains. Most of the studies relating to FAK function have focused on the C-terminal domain. FAK-related non-kinase (FRNK) is a product of the FAK gene generated by an alternative transcriptional start site within an intron [26]. The protein product of the FRNK transcript corresponds to the C-terminal domain of FAK [27]. FRNK contains the focal adhesion targeting sequence [28] and localizes to focal contacts where it acts as a negative regulator of FAK function [29]. The C-terminal domain of FAK is a crucial mediator of its localization and survival function in tumours. When the same C-terminal region of the human FAK protein was ectopically expressed in BT-474 breast cancer cells, it caused irreversible loss of adhesion and viability, as well as a corresponding decrease in FAK tyrosine phosphorylation [20]. In a similar fashion, adenovirus-mediated expression of the C-terminus of FAK induced apoptosis in breast cancer cells [30,31].

The N-terminal domain of FAK has been implicated in cell signalling, largely through its stimulation by integrin activation. This domain contains the putative binding site for integrins, and the FAK N-terminus binds to peptides mimicking the β1-integrin cytoplasmic domain [32], suggesting that integrins could interact directly with FAK. However, this interaction has not been demonstrated *in vivo*, and it is also possible that FAK binds indirectly to integrins through an interaction between the FAK C-terminus and talin ([33] and reviewed by Cary and Guan [7]). The N-terminal region of FAK contains the major autophosphorylation site, Tyr[397] [34], which would point to an important role of this domain in the functions of FAK. Phosphorylated Tyr[397] creates a binding site for the Src homology 2 (SH2) domain of Src-family kinases [34], including Fyn [13], phosphoinositide 3-kinase [35], phospholipase C-γ1 [36] and Grb7 [37]. The proline-rich sequence at amino acids 368–377 (RALPSIPKAN), proximal to the Tyr[397], stabilizes the interaction of FAK with Src through an SH3 domain interaction [36].

Abbreviations used: Ad-LacZ, adenovirus expressing *lacZ* gene; FAK, focal adhesion kinase; FAK-NT, FAK N-terminus; Ad-FAK-NT, adenovirus expressing the FAK-NT construct; FRNK, FAK-related non-kinase; ffu, focus-forming unit; GFP, green fluorescent protein; Grb, growth-factor-receptor-bound protein; HA, haemagglutinin; SH, Src homology domain; Y397F, Tyr[397] → Phe.

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In the present study, we have examined the effect of expressing the N-terminus of FAK (FAK-NT) in human breast cancer cells. Transient transfection of FAK-NT caused an early and transient rounding of the breast cancer cells, while adenoviral infection of FAK-NT led to cellular rounding, loss of FAK tyrosine phosphorylation and apoptosis. The same pathway was not affected in non-transformed MCF10A breast epithelial cells, suggesting that the N-terminal region of FAK directs an adhesion response that is required for breast cancer cell survival, but not for non-transformed breast epithelial cells.

EXPERIMENTAL

Cell lines and culture

BT-474 human breast carcinoma cells were obtained from the A.T.C.C. BT-474 cells were cultured in RPMI 1640 medium (Gibco BRL) supplemented with 10 μg/ml insulin and 10 % fetal bovine serum. MCF7 cells were cultured in Eagle's minimal essential medium containing 10 % fetal bovine serum, 10 μg/ml insulin, 1 mM sodium pyruvate and 0.1 mM non-essential amino acids. Cells were transfected with pUse-Src, selected for the pUse vector and maintained in medium containing 400 μg/ml of G418.

Plasmid constructions

The 1268-bp fragment corresponding to the FAK-NT sequence was amplified from the full-length FAK cDNA by PCR. The primers used were 5′-ACCGGTACCTCATGGCAGCTG-CTTACCTTG-3′ and 5′-CATGGGCCCGTTCTATTCTTTCTC-TTTG-3′. The amplified product was digested with *Kpn*I and *Apa*I, and ligated into the pEGFP-N1 plasmid (ClonTech, Palo Alto, CA, U.S.A.). DNA was purified using a Qiagen plasmid purification kit (Qiagen, Chatsworth, CA, U.S.A.), and the sequence identity was confirmed by *Taq* FS Dye Terminator sequencing (Automated DNA Sequencing Facility, University of North Carolina at Chapel Hill). The FAK-NT sequence used in the present study contains the FAK N-terminus plus six amino acids of the FAK kinase domain.

The $Tyr^{397} \rightarrow Phe$ (Y397F) substitution was created from a full-length pcDNA3-FAK construct and was kindly provided by Dr Xiong Li and Dr Shelton Earp (Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill). The Y397F FAK-NT mutant was generated from this construct using the PCR protocol described above and named FAK-Y397F/NT. After confirming the identity of the sequence, FAK-Y397F/NT was cloned into the pEGFP-N1 vector. The plasmid pcDNA3 containing the full-length haemagglutinin (HA)-tagged FAK was used in the transient transfection experiments to over-express exogeneous $p125^{FAK}$ in BT-474 cells.

The pShuttle HA-FAK-NT was constructed by subcloning the PCR-amplified FAK-NT from pcDNA3 HA-FAK into the *Kpn*I and *Hin*dIII sites of the pShuttle-CMV vector (Virus Vector Core Facility, University of North Carolina at Chapel Hill). The adenoviral pShuttle HA-FAK-NT was constructed by subcloning the PCR-amplified FAK-NT from pcDNA3 HA-FAK into the *Kpn*I and *Hin*dIII sites of the pShuttle-CMV vector (Virus Vector Core Facility). The recombinant virus containing FAK-NT, called Ad-FAK-NT, was used for adenoviral infections as described previously [30].

The pUse/c-*src* construct (Upstate Biotechnology, Lake Placid, NY, U.S.A.), containing an activating Y529F mutation of murine c-Src, was stably transfected using standard techniques. These cells were characterized and shown to over-express c-Src and possess Src-kinase activity (results not shown). In addition, the pUse host vector was stably transfected as a control.

Transfections and infections

For transient transfection, 1×10^5 cells were plated on to coverslips in six-well plates or 1.5×10^6 were plated on to 100-mm tissue culture dishes for 24 h. Cells were then transfected with a 1 : 3 ratio of DNA and LIPOFECTAMINE™ (Life Technologies) for 8 h. The transfection mixture was removed and replaced with medium containing serum at various time intervals. Cells on coverslips were washed with PBS, fixed in 2 % paraformaldehyde and mounted on slides. Cell morphology was observed by fluorescence microscopy; round transfected cells were counted and photomicrographs were obtained using a Zeiss fluorescence microscope.

For infections, cells were plated at 1×10^6 per 100-mm culture plate for 24 h and infected with Ad-FAK-NT or Ad-LacZ control adenovirus, carrying the *lacZ* gene, expressing β-galactosidase [kindly provided by Dr J. Samulski (Virus Vector Core Facility)]. Cells were infected at different doses and expression of FAK-NT was verified by Western blotting using anti-HA and anti-FAK antibodies.

Antibodies

The anti-[green fluorescent protein (GFP)] monoclonal antibody was purchased from ClonTech, and the anti-FAK monoclonal antibody 4.47, recognizing an epitope within amino acids 1–423 of FAK, and the kinase domain-specific antibody were purchased from Upstate Biotechnology, whereas the anti-(phospho-Tyr^{397}-FAK) antibody was from Biosource International. The other antibodies used in this study were the anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology), the monoclonal anti-vinculin (clone VIN-11–5) antibody (Sigma), the anti-HA antibody HA11 (BabCO, Richmond, CA, U.S.A.), and monoclonal antibodies to β-actin (Sigma) and inactive caspase-3 (Transduction Laboratories).

Immunofluorescence microscopy

Cells were plated on to uncoated coverslips in six-well culture plates in serum-containing medium for 24 h, transfected with the GFP or GFP-FAK-NT constructs, as described above, and then stained for vinculin 16–24 h later. Rhodamine-conjugated anti-mouse secondary antibodies were used where indicated. Conditions for immunofluorescence and measurement of apoptosis have been described previously [30].

Immunoprecipitation and Western-blot analysis

BT-474 cells transfected with the GFP or GFP-FAK-NT constructs were harvested with trypsin and EDTA, washed twice in Hanks balanced salt solution, and lysed in suspension in 1 % Nonidet P-40 lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl and 0.5 mM EDTA) containing 0.1 mM Na_3VO_4 and a cocktail of protease inhibitors. The protein concentrations of the lysates were determined using the bicinchoninic acid assay (Pierce Chemical, Rockford, IL, U.S.A.). Equal amounts of protein were immunoprecipitated with anti-FAK rabbit polyclonal antibody in the presence of Protein G Plus/Protein A–agarose (Oncogene Research Products, Cambridge, MA, U.S.A.).

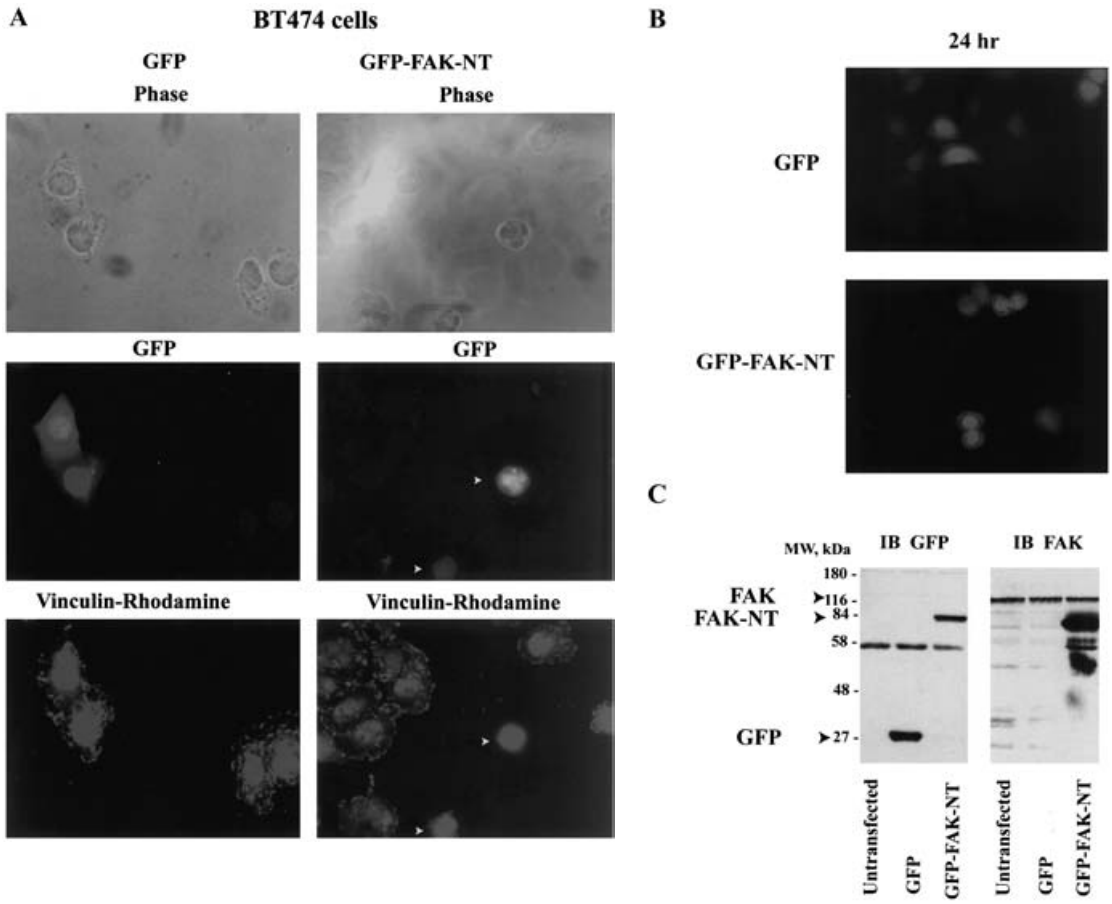


Figure 1 Morphological rounding induced by expression of FAK-NT in BT-474 breast cancer cells

(**A**) Cells were transiently transfected with GFP or GFP-FAK-NT constructs, fixed and observed by fluorescence microscopy. At the time point of 24 h, GFP-FAK-NT transfectants showed a rounded morphology. Left panels, GFP-transfected cells; right panels, GFP-FAK-NT-transfected cells; upper panels, phase contrast image; middle panels, GFP-expressing cells under the FITC filter; lower panels, vinculin–Rhodamine immunostaining. Arrows indicate round FAK-NT-expressing cells with lost focal adhesions detected by the absence of vinculin in contrast with the control GFP-expressing cells. (**B**) FACS-sorted cells transfected as in (**A**). The selected fluorescent population was replated on coverslips in the serum containing medium for an additional 24 h. FAK-NT cells remained rounded compared with the GFP-expressing control cells. (**C**) Western-blot analysis of FAK-NT expression. BT-474 cells were lysed 24 h after transfection with GFP or GFP-FAK-NT plasmids. Equal amounts of cell lysate were subjected to Western-blot analysis with anti-GFP or anti-(FAK-NT) monoclonal antibodies. The level of FAK expression remained relatively constant at 48 h.

Immunoprecipitated samples were resolved by SDS/PAGE (7.0 % gel) and transferred on to PVDF membrane. Blots were blocked with a 3 % BSA/0.1 % Tween/TBS solution and blotted with anti-phosphotyrosine monoclonal antibody (4G10). Blots were also stripped and reprobed with an anti-(FAK-NT) monoclonal antibody to control for loading. The immunoreactive bands were detected with the ECL® detection system.

Cell rounding assay

Cells were transiently transfected with the GFP or GFP-FAK-NT constructs, fixed at different time points after transfection and observed by fluorescent microscopy. The percentage of rounded cells was determined by dividing the number of rounded GFP- or GFP–FAK-NT-expressing (green) cells by the total number of GFP-expressing green cells. For some experiments, cells were harvested 24 h after transfection and sorted by FACS analysis. The selected fluorescent population of cells was replated in medium with 10 % FBS for 24 h and observed for rounded morphology.

RESULTS

For these experiments, the first 423 amino acids of FAK were fused in frame with GFP in the plasmid pEGFP-N1/FAK-NT. BT-474 human breast cancer cells were transiently transfected with pEGFP-N1 vector (GFP) or pEGFP-N1/FAK-NT plasmid (GFP-FAK-NT), and then observed under the fluorescence microscope after incubation at 37 °C for various time periods. The transfection efficiency was consistently 20–25 % for cells transfected with either the GFP or GFP-FAK-NT construct. From 2.5 to 24 h following transfection, GFP–FAK-NT-expressing BT-474 cells developed a rounded morphology (Figure 1A and Table 1). Rounded GFP-FAK-NT-transfected cells lost focal adhesion formation, as detected by vinculin staining (Figure 1A, right lower panel), in contrast with GFP-expressing cells (Figure 1A, left lower panel). To increase the number of transfected cells, we analysed the morphology of GFP- and GFP–FAK-NT-expressing cells sorted by FACS and 24 h post-transfection. As before, we observed rounding among GFP–FAK-NT-expressing cells compared with GFP-expressing cells (Figure 1B). Expression of the 80 kDa GFP–FAK-NT protein was assessed by Western blotting using both anti-GFP and anti-FAK N-terminal antibodies

Table 1 Induction of cellular rounding in BT-474 breast cancer cells by GFP–FAK-NT

Cells were transfected with the GFP or GFP-FAK-NT constructs and the percentages of round transfected cells were determined at different time points post-transfection by fluorescence microscopy. Results are expressed as the means ± S.D. *$P < 0.05$, **$P < 0.01$; Student's *t* test. All experiments were repeated more than five times with the same result, and the representative experiments are shown in the Table. For each experiment more than 100 cells were counted in more than three independent fields. The percentage of rounded cells was determined by dividing the number of round GFP- or GFP-FAK-NT-expressing (green) cells by the total number of green cells, which expressed GFP plasmids.

	Rounded cells (%)			
	Experiment 1	Experiment 2		
Plasmids	2.5 h	4 h	8 h	24 h
GFP	14.6 ± 8.9	19 ± 6.0	29.6 ± 8.8	22.5 ± 6.4
GFP–FAK-NT	43.3 ± 9.8*	36.4 ± 9.3*	51.6 ± 11.0*	54.7 ± 12.4*

(Figure 1C). FAK-NT-mediated rounding was not specific to BT474 cells, because GFP–FAK-NT induced rounding in 40 ± 11 % of SK-BR-3 breast carcinoma cells. By comparison, GFP alone induced 15 ± 6 % rounding in these cells.

To rule out the possibility that GFP itself contributed to the effect of FAK-NT, we made an HA-tagged FAK-NT construct by subcloning FAK-NT into the pShuttle-CMV expression vector. We transfected the pShuttle HA-FAK-NT into BT-474 cells, and the cells expressing pShuttle HA-FAK-NT showed a similar percentage of rounding (38 ± 10 %) as that induced by GFP–FAK-NT, demonstrating that cellular rounding induced by GFP–FAK-NT was not due to the GFP fusion partner.

We hypothesized that FAK-NT-mediated cell rounding resulted from competition for binding sites between endogenous FAK and exogenous FAK-NT. If so, over-expression of $p125^{FAK}$ might reverse the rounding effect induced by FAK-NT. To test this, BT474 cells were transiently co-transfected with the GFP-FAK-NT and pcDNA3-HA-FAK constructs at a 1 : 3 ratio. As expected, FAK over-expression prevented GFP–FAK-NT-mediated cellular rounding, whereas cells transfected with GFP-FAK-NT construct and a control plasmid rounded (Figure 2A). Cells expressing HA-tagged FAK and either GFP or GFP–FAK-NT (Figure 2B, middle panels) were stained with an antibody to HA, and in both cases exogenous full-length FAK protein localized to the cell periphery (Figure 2B, right panels). Furthermore, FAK over-expression preserved normal spread cell morphology and normal focal adhesion organization (Figure 2B, left panels). Expression of exogenous HA-tagged FAK and GFP-FAK-NT plasmids was also demonstrated by Western-blot analysis (Figure 2C).

Because FAK and c-Src physically associate through a site in the N-terminus of FAK, we tested whether BT-474 cells expressing a constitutively active mutant of c-Src were sensitive to the transient rounding induced by FAK-NT (Figures 2D–2F). BT-474 cells were transfected with the pUse vector or pUse containing activated murine c-Src, and stably over-expressing clones were identified (Figure 2F). Whereas the pUse vector control cells transfected with the constructs for GFP-FAK-NT or GFP-FAK-Y397F-NT mutant showed significant rounding (Figure 2D), the activated c-Src-over-expressing cells transfected with GFP-FAK-NT vector had a very low percentage of rounding (Figure 2D), similar to the level shown by BT-474 cells transfected with GFP vector. Expression of both the GFP–FAK-NT and GFP–FAK-NT-Y397F proteins was verified by Western blotting (Figure 2E), and we also detected absence of Tyr^{397} phosphorylation in the GFP–FAK-NT-Y397F mutant (results not shown). Thus activated c-Src is capable of conferring resistance to the induction of the rounded phenotype induced by FAK-NT and FAK-NT-Y397F vectors in BT-474 cells.

The N-terminal Tyr^{397} of FAK is a binding site for a number of kinase SH2 domains, and the interaction of the Src family of kinases with this site has been extensively characterized [34,38]. Mutation of Tyr^{397} to Phe in the GFP-FAK-NT construct did not affect FAK-NT-mediated cellular rounding, and the ability of GFP–FAK-NT-Y397F to induce rounding was completely suppressed by activated Src (Figure 2D). We conclude that FAK-NT does not affect cellular adhesion exclusively through Tyr^{397}-mediated interactions, and that Src does not suppress FAK-NT-mediated cellular rounding by binding to Tyr^{397}.

Transient transfections are limited by the low efficiency of transfection (5–27 % for most of these experiments) and the toxicity of the transfection procedure. To increase transfer efficiency, we developed an adenovirus directing expression of FAK-NT fused in frame with an HA-epitope tag sequence. Infection of BT474 cells with Ad-FAK-NT caused cellular rounding similar to the transient transfection procedure used above (Figure 3A, right upper and middle panels), in contrast with cells infected with Ad-LacZ (Figure 3A, left upper and middle panels).

FAK generally localizes to focal adhesions in BT474 cells, with a significant fraction localized in a diffuse manner throughout the cytoplasm and nucleus. This localization was not affected by the Ad-LacZ control virus (Figure 3A, lower left panel). In contrast, infection of BT474 cells with Ad-FAK-NT displaced FAK from focal adhesions (Figure 3A, lower right panel). Intriguingly, FAK-NT appeared to have significant nuclear localization in BT474 cells (Figure 3A, middle panel), consistent with previous studies in other cell lines [39–41].

Breast cancer cell lines are genetically unstable and vary greatly in biological responses. For that reason, we tested the MCF-7 breast cancer cell line for rounding after infection with Ad-FAK-NT. As in BT474 cells, MCF-7 cells infected with Ad-FAK-NT demonstrated localization of FAK-NT to the nucleus with subsequent rounding and detachment from the dish (Figure 3B, right upper and middle panels) in contrast with Ad-LacZ-infected MCF-7 cells (Figure 3B, left upper and middle panels). Infection with Ad-FAK-NT led to displacement of FAK from focal adhesions (Figure 3B, lower right panel). BT474 and MCF-7 cells infected with Ad-FAK-NT expressed high levels of HA-tagged FAK-NT protein (Figure 3C).

Displacement of FAK from focal adhesions led to the degradation of FAK in BT474 cells infected with Ad-FAK-NT (Figure 4A, upper panel). In MCF-7 cells, FAK-NT-mediated regulation of FAK was more complex. At the 125 focus-forming unit (ffu)/cell viral dose, FAK became dephosphorylated and FAK was partially degraded (Figure 4B), and at higher doses FAK was degraded (Figure 4B). Because Tyr^{397} is a key binding site for a number of proteins to the N-terminus of FAK, we analysed the phosphorylation of Tyr^{397} following Ad-FAK-NT infection, and as expected, FAK Tyr^{397} became dephosphorylated and FAK was degraded with increased doses of Ad-FAK-NT (Figure 5A, upper and middle panels) in BT474 cells and similarly in MCF-7 cells (Figure 5B, upper and middle panels).

Degradation of FAK accompanies apoptosis and may contribute to apoptotic progression. Cleaved forms of FAK appeared in a time- and adenoviral dose-dependent manner, following infection with Ad-FAK-NT in BT-474 cells (Figures 4A and 5A, middle panels) and in adenoviral dose-dependent manner in MCF-7 cells (Figures 4B and 5B, middle panels). We found that Ad-FAK-NT led to activation of caspase-3 in BT474 cells, a key step in apoptotic progression (Figure 5A, third panel) and caused an

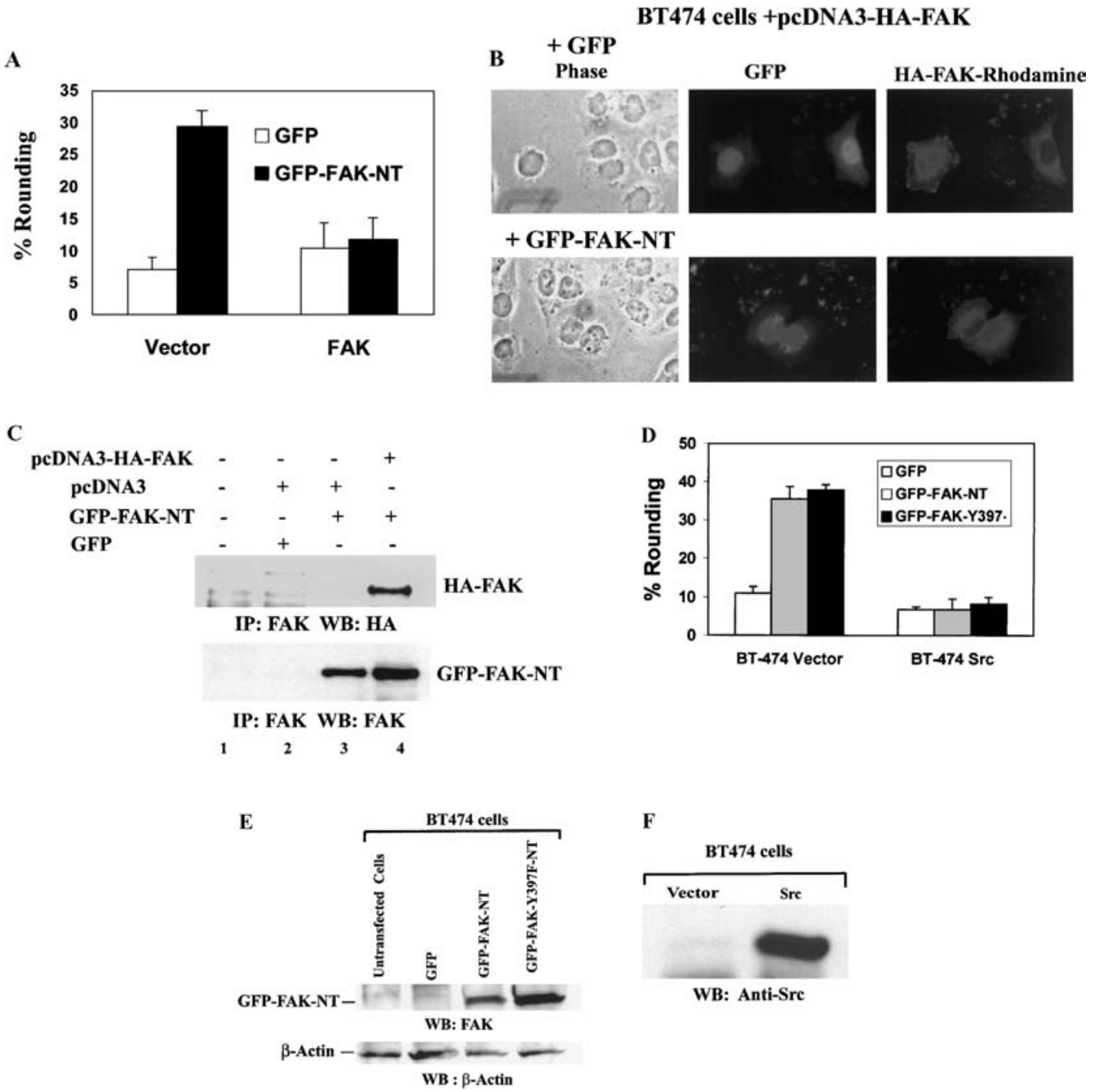
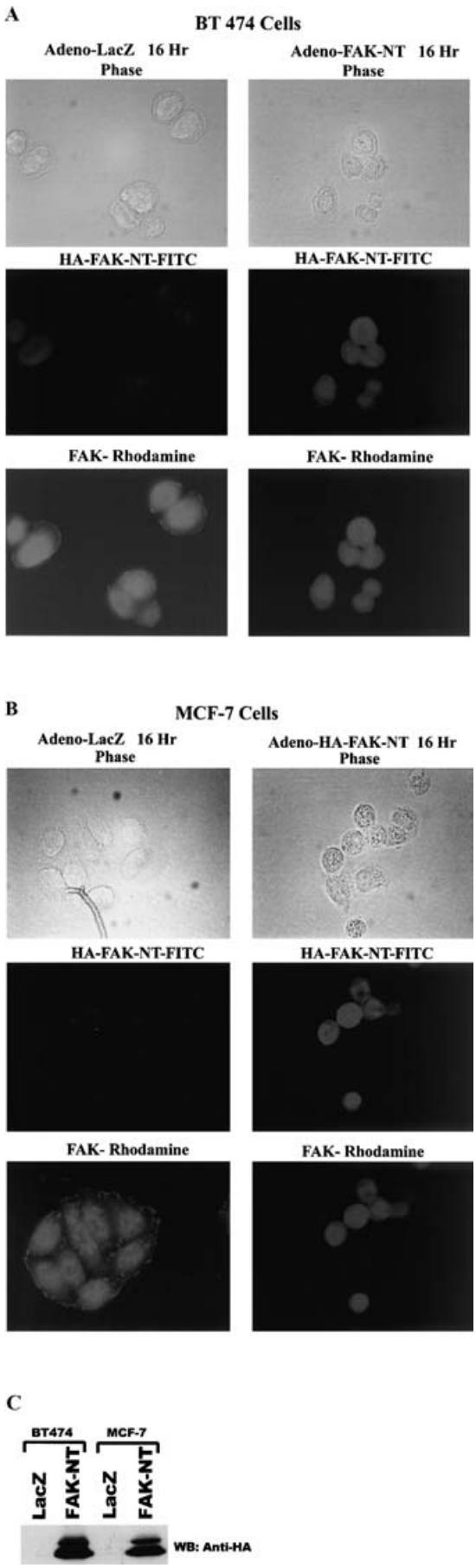


Figure 2 Reversal of FAK-NT-mediated cell rounding by over-expression of full-length FAK or activated c-Src

BT474 cells were transfected with a GFP control vector (open bars) or GFP-FAK-NT construct (closed bars) and observed microscopically 24 h later. (**A**) Cellular rounding mediated by GFP–FAK-NT was reversed by over-expression of the full-length FAK protein by co-transfection with the pcDNA3 HA-FAK 24 h post-transfection. All experiments were done in triplicate, and repeated >3 times with the similar results, as described in Table 1. The average percentage of rounding $\pm$ S.D. are shown. (**B**) Expression of HA–FAK and GFP proteins. BT474 cells were transfected with HA-tagged FAK-pcDNA3 plasmid and either GFP (upper panels) or GFP-FAK-NT (lower panels) vectors. Left panels, phase contrast images of cells. Expression of the GFP proteins was assessed by GFP fluorescence (middle panels) or immunofluorescence with an anti-HA antibody (right panels). Expression of the full-length HA-FAK proteins suppressed FAK-NT-mediated cell rounding (phase contrast images). (**C**) Western-blot analysis (WB) of GFP–FAK-NT and HA–FAK protein expression. Presence of transfected plasmid is indicated by plus ($+$) and absence by minus ($-$). Untransfected cells are shown in lane 1. Cells were transfected either with GFP $+$ pcDNA3 control vector (lane 2), GFP-FAK-NT $+$ pcDNA3 vector (lane 3) or GFP-FAK-NT $+$ pcDNA3-HA-FAK vectors (lane 4). Cell lysates were analysed 24 h post-transfection by immunoprecipitation (IP) with anti-FAK antibody, and probed first with anti-HA tag antibody (upper panel), then stripped and re-probed with anti-FAK antibody (lower panel). Transfected cells expressed high levels of HA–FAK and GFP–FAK proteins 24 h after transfection. (**D**) Transfection of GFP-FAK-NT into a BT474 line over-expressing activated c-Src did not induce cellular rounding. Because FAK and Src associate physically via the Tyr^{397} of FAK, the GFP-FAK-NT-Y397F was tested to see whether it induced cellular rounding. Transfection of the GFP-FAK-NT-Y397F mutant (closed bars) caused cellular rounding to a similar extent found in the wild-type protein (grey bars), as compared with GFP protein (open bars), and rounding induced by the GFP-FAK-NT-Y397F construct was completely reversed by activated Src. Rounding was analysed at 24 h post-transfection in several independent experiments with the same result, as described in Table 1. The representative experiment is shown. The results are expressed as the average percentage of rounding $\pm$ S.D. (**E**) Expression of GFP–FAK-NT and GFP–FAK-NT-Y397F by Western blotting. At 24 h post-transfection, BT474 cells were collected and analysed with an anti-FAK antibody. Equal protein loading was analysed with anti-(β-actin) antibody. (**F**) Expression of activated c-Src was examined by Western blotting with an anti-Src antibody. BT-474-Src cells expressed high levels of Src protein. Equal amounts of protein were loaded on to the gel.



increase in apoptosis (Table 2). As in BT474 cells, there was a marked increase in the percentage of apoptotic MCF7 cells following Ad-FAK-NT infection, but not in control Ad-LacZ-infected cells (Table 2). We conclude that the FAK N-terminus regulates adhesion and suppresses apoptosis in multiple breast cancer cell lines.

Because FAK-NT over-expression led to loss of adhesion in breast cancer cells, we determined whether this was a general property of breast epithelial cells. MCF10A cells are derived from breast with fibrocystic disease and are a relatively normal cell line. Infection of MCF10A cells did not cause cellular rounding, even though FAK-NT was readily detectable (Figure 5C). MCF10A cells infected with Ad-FAK-NT maintained normal levels of phosphorylation of Tyr^{397} (Figure 5C, upper panel) and FAK was not degraded (Figure 5C, second panel).

In addition, we did not detect any increase in the percentage of apoptotic cells in MCF10A cells (Table 2) or activation of caspase-3 following infection with Ad-FAK-NT (Figure 5C, third panel). Interestingly, FAK-NT underwent a different degree of processing in MCF10A (Figure 5C, second panel) compared with BT474 and MCF-7 cells (Figures 5A and 5B respectively, middle panels). It is possible that the altered electrophoretic mobility of FAK-NT in these cells underlies its distinct functions in non-transformed and cancer cell lines. Normal MCF-10A cells infected with Ad-FAK-NT remained spread (Figure 5D, upper panels) with FAK localized throughout the cytoplasm (Figure 5D, lower panels) and at the focal adhesions (results not shown). FAK-NT was localized in a diffuse pattern mainly in the cytoplasm (Figure 5D, middle panels). We conclude that FAK-NT serves in the maintenance of adhesion and apoptosis in tumour cells, but is not required for adhesion in non-transformed breast epithelial cells. We also present a model of FAK-NT-induced apoptosis in cancer cell lines (Scheme 1).

DISCUSSION

We have shown that over-expression of the FAK N-terminal domain induces rounding, detachment and apoptosis in breast cancer cell lines. This finding coincides with our previous work with the FAK C-terminal domain in which over-expression of the FAK C-terminus caused loss of adhesion [42] and apoptosis [30,31] in breast cancer cell lines. These results are all consistent with a role of FAK in cell survival signalling [22,23,30,42–46].

Survival signalling is a central pathway in cancer progression. As epithelial cells proliferate and begin to invade their surrounding substratum, it is necessary to suppress the apoptotic signals associated with loss of cell–cell contact. FAK is a key mediator of survival signalling, perhaps functioning by amplifying a surrogate

Figure 3 Adenoviral transduction of FAK-NT induces cellular rounding

(**A**) BT474 cells were infected with a control adenovirus (Ad-LacZ) or an adenovirus containing FAK-NT (Ad-FAK-NT) at a dose of 125 ffu/cell and grown for 16 h. Infected cells expressing the HA-tagged FAK-NT (HA immunofluorescence on the right middle panel) underwent cellular rounding (right upper panel), while endogenous FAK was displaced from the focal adhesions (demonstrated with FAK kinase domain-specific antibody, right lower panel). Cells infected with a control adenovirus did not undergo rounding (left upper panel), were not immunoreactive for HA (left middle panel), and expressed FAK at the focal adhesion contacts at the cell periphery (left lower panel). (**B**) Infection of MCF7 breast cancer cells with Ad-FAK-NT causes cellular rounding. MCF7 cells were infected with 500 ffu/cell of Ad-LacZ control or Ad-FAK-NT adenoviruses and grown for 16 h. Cells were visualized by bright field (upper panels), immunofluorescence with an antibody to HA (middle panels), and a FAK kinase domain-specific antibody (lower panels). Infection with Ad-FAK-NT caused cellular rounding (right upper panel) that corresponded with HA–FAK-NT expression (right middle panel), and displacement of endogenous FAK from focal adhesions (compare left and right lower panels). (**C**) Western blot (WB) showing expression of HA–FAK-NT protein following infection into BT474 and MCF7 breast cancer cell lines.

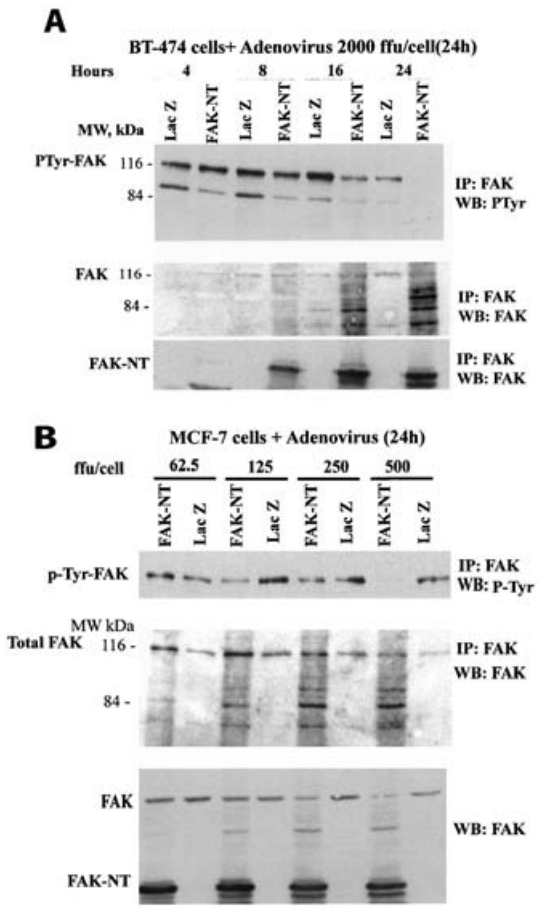


Figure 4 FAK tyrosine phosphorylation is diminished in cells infected with Ad-FAK-NT

(**A**) FAK was immunoprecipitated from BT474 cells infected with the Ad-FAK-NT adenovirus and analysed for expression (middle panel) and total tyrosine phosphorylation (upper panel). FAK tyrosine phosphorylation decreased dramatically within 24 h of infection, while FAK phosphorylation was much less affected in cells expressing a control Ad-LacZ adenovirus (Lac Z lanes). Decreased FAK tyrosine phosphorylation correlated with degradation of the FAK protein (middle panel). During the same time course, expression of the exogenous FAK-NT protein increased (lower panel). (**B**) FAK was immunoprecipitated from MCF7 cells infected with increasing amounts of Ad-LacZ control or Ad-FAK-NT adenoviruses and analysed by Western blotting for FAK expression (middle panel) or tyrosine phosphorylation (upper panel). Ad-FAK-NT expression led to loss of FAK tyrosine phosphorylation (upper panel) and the appearance of lower molecular mass FAK degradation products (middle panel). A Western blot of FAK demonstrates FAK-NT expression with increasing doses of adenovirus (lower panel). IP, immunoprecipitation; MW, molecular-mass markers; PTyr, phosphotyrosine; WB, Western blot.

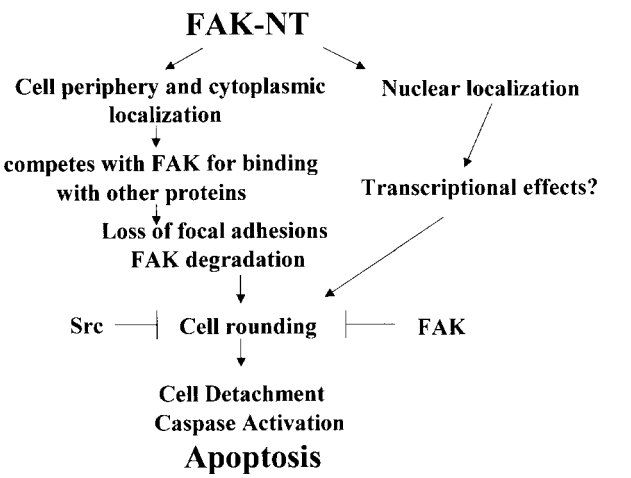
adhesion signal, even as invading cells are deprived of cell–cell contact. Indeed, we have shown that cultured breast cancer cells deprived of adhesion to a culture dish are viable, but that attenuation of FAK function leads to a dramatic increase in apoptosis [30]. Whereas our previous work focused on the role of the FAK C-terminus in survival signalling [30,31,42], the present work indicates an equally important and novel role for the FAK-NT, and the role of FAK-NT in apoptosis is proposed in Scheme 1.

The molecular features of FAK required for survival signalling are not known. Richardson et al. [47] analysed various mutants of FAK for their ability to suppress loss of adhesion associated with over-expression of the FRNK variant of the FAK C-terminal domain. FAK over-expression suppressed loss of adhesion due to FRNK expression, and this suppression was complete even in kinase-deficient FAK mutants [47]. In contrast, loss of the

Table 2 Induction of apoptosis by infection with Ad-FAK-NT

Cells were infected with Ad-Lac-Z and Ad-FAK-NT (at a dose of 500 ffu/cell), and detection of apoptosis was performed by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) assay. The percentage of apoptotic cells was determined by dividing the number of apoptotic cells by the total number of cells following 23 h of adenoviral transduction. Apoptotic cells were counted in three independent fields, where 100 cells per field were counted. The experiments were performed two times with the same result. Results are expressed as the means ± S.D. Infection of breast cancer cells with Ad-FAK-NT caused significant increase of apoptosis.

	Apoptotic cells (%)	
Cell line	Ad-LacZ	Ad-FAK-NT
BT-474	0	56 ± 3.6
MCF-7	0	58 ± 4.0
MCF10A	0	0



Scheme 1 Model for FAK-NT induced rounding and apoptosis in breast cancer cell lines

Tyr^{397} binding site negated the suppressive activity of FAK, suggesting that Src binding was required for restoring adhesion to FRNK-expressing cells [47]. Richardson et al. [47] concluded that FAK acts as a 'flexible adaptor', bringing together the Src tyrosine kinase with its substrate paxillin [47].

Our data are consistent with the 'flexible adaptor' model because both the N- and C-terminal domains are capable of disrupting the FAK survival function.

The N-terminus of FAK binds to a number of key signalling proteins through its N-terminus, including Src [34], Grb7 [37], phosphatidylinositol 3-kinase [35], and phospholipase C-γ1 [48]. All of these proteins bind to Tyr^{397} upon phosphorylation, and Tyr^{397} is required for FAK-mediated cell spreading [47,49]. However, our results differ from those of Richardson et al. [47] because mutation of the Tyr^{397} to Phe did not affect the ability of FAK-NT to induce cellular rounding. We conclude that proteins other than those which bind to Tyr^{397} are important for FAK-NT-induced cellular rounding, or that a subset of proteins which bind to the FAK N-terminus can still bind in the absence of Tyr^{397}. In the latter case, one possible binding partner for FAK-NT is the Src family of kinases, which also associate with an SH3 binding site at amino acids 368–377 of FAK [36].

FAK is anchored to focal adhesions through a sequence in the C-terminal region called the focal adhesion targeting sequence. For that reason, we were surprised that FAK was displaced from

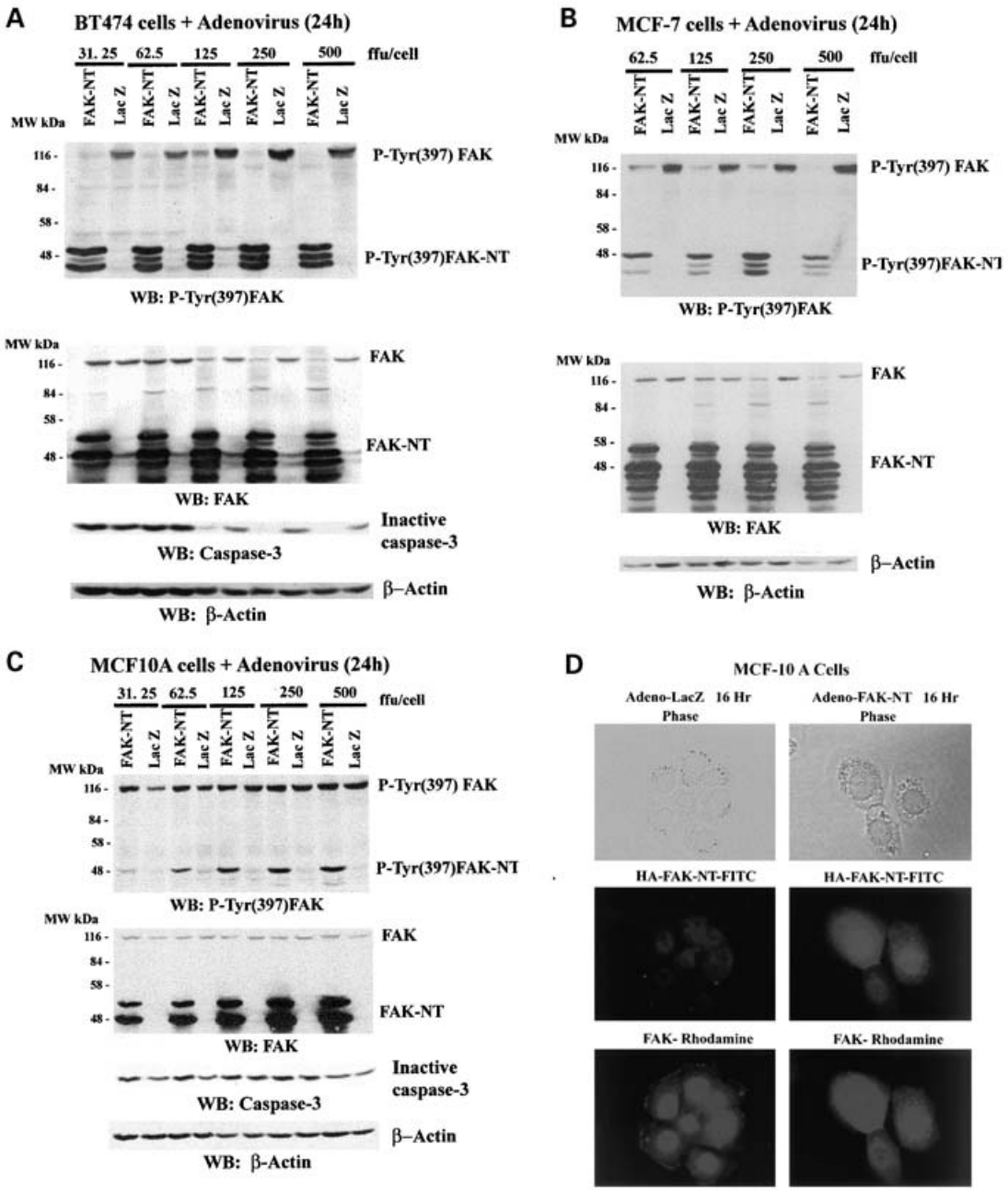


Figure 5 Decreased tyrosine phosphorylation of Tyr^{397} of FAK corresponds with FAK degradation and increased caspase-3 activation in breast cancer cell lines

(**A**) Elevated dosage of Ad-FAK-NT adenovirus causes loss of FAK Tyr^{397} phosphorylation (125 kDa band, upper panel), FAK degradation (middle panel, 80 kDa band), and disappearance of the inactive caspase-3 isoform (third panel). All analyses were Western blots of BT474 cells infected with increasing doses of Ad-LacZ or Ad-FAK-NT, as indicated, probed with antibodies to phosphorylated FAK Tyr^{397}, FAK, inactive caspase-3 or β-actin. (**B**) Infected MCF7 cells were lysed and probed with a phospho-specific FAK Tyr^{397} antibody, demonstrating loss of Tyr^{397} phosphorylation in Ad-FAK-NT-infected cells, but not in Ad-LacZ-infected cells. (**C**) Infection of MCF10A breast epithelial cells with the Ad-FAK-NT adenovirus. MCF10A cells were infected with increasing doses of the Ad-LacZ control or Ad-FAK-NT adenoviruses as indicated and lysed 24 h post-infection. Lysates from infected cells were analysed for FAK Tyr^{397} tyrosine phosphorylation (upper panel), FAK and FAK-NT expression (second panel), caspase-3 inactive form degradation (third panel) and β-actin expression (lower panel). The Ad-FAK-NT adenovirus directed strong expression of the FAK-NT protein (two upper panels), but did not affect cellular adhesion, FAK expression (second panel), FAK Tyr^{397} phosphorylation (upper panel), or caspase-3 inactive form activation (third panel). (**D**) MCF10A cells were infected with a control adenovirus (Adeno-LacZ) or an adenovirus containing FAK-NT (Adeno-FAK-NT) at a dose of 500 ffu/cell and grown for 16 h. Infected cells expressing the HA-tagged FAK-NT (HA immunofluorescence, right middle panel) did not undergo cellular rounding (right upper panel) and endogenous FAK was cytoplasmic [demonstrated with FAK kinase domain-specific antibody (right lower panel)]. FAK-NT localized mainly in cytoplasm (right middle panel). Cells infected with a control adenovirus did not round (left upper panel), were not immunoreactive for HA (left middle panel), and expressed FAK at the focal adhesions at the cell periphery (left lower panel).

focal adhesions by FAK-NT. A recent report [50] has found a role for FAK-NT in directing localization of FAK to focal adhesions. Our results are consistent with the model that there is a second focal adhesion-targeting domain in the FAK-NT, and that disruption of this domain leads to displacement of FAK from focal adhesions and cancer cell apoptosis.

FAK is over-expressed in a broad variety of tumours, including breast tumours, suggesting a distinct role for FAK in tumorigenesis. Indeed, expression of the FAK C-terminal domain induced apoptosis in tumour cells, but not in non-transformed breast epithelial cells [30]. Similarly, FAK-NT induced apoptosis in cancer-derived cell lines, but not in non-transformed cells. We conclude that the essential function of FAK in tumours is not exclusively due to the C-terminus, but to the entire protein. Finally, these results support our previous observations [42] that epithelial tumour cells are more sensitive to the effects of interrupting FAK signalling than their non-transformed counterparts. Indeed non-transformed MCF-10 breast epithelial cells did not demonstrate the round phenotype unless they were *ras*-transformed (results not shown). Cancer cells often have morphological abnormalities that correlate with detachment and apoptosis, leading to adhesion independence and invasiveness, but normal cells can suppress apoptosis, not only by adhesion to the extracellular matrix, but also through distinct cell spreading morphology and integrity of cytoskeleton differences, as was shown in MCF10A cells [51]. These cells required actin depolymerization agents in addition to other apoptotic stimuli to cause apoptosis [51], which is in contrast with cancer cell lines. These results demonstrate an important role for the entire FAK protein in suppressing apoptosis in human tumour cells, as shown in Scheme 1. Whereas the C-terminus appears to localize FAK and mediate some aspects of tumour cell survival, the N-terminus also appears to contribute to the adhesive and apoptosis-suppressive functions of FAK. As cells become transformed and migrate to distant sites, both the N- and C-terminal domains may serve as excellent targets for pharmaceutical intervention.

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REFERENCES

1 Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B. and Parsons, J. T. (1992) pp125^{FAK} a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc. Natl. Acad. Sci. U.S.A. **89**, 5192–5196
2 Zachary, I. (1997) Focal adhesion kinase. Int. J. Biochem. Cell Biol. **29**, 929–934
3 Kanner, S. B., Reynolds, A. B., Vines, R. R. and Parsons, J. T. (1990) Monoclonal antibodies to individual tyrosine-phosphorylated protein substrates of oncogene-encoded tyrosine kinases Proc. Natl. Acad. Sci. U.S.A. **87**, 3328–3332
4 Cobb, B. S., Schaller, M. D., Leu, T. H. and Parsons, J. T. (1994) Stable association of pp60^{src} and pp59^{fyn} with the focal adhesion-associated protein tyrosine kinase, pp125^{FAK}. Mol. Cell. Biol. **14**, 147–155
5 Kornberg, L. J., Earp, H. S., Turner, C. E., Prockop, C. and Juliano, R. L. (1991) Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of β1 integrins. Proc. Natl. Acad. Sci. U.S.A. **88**, 8392–8396
6 Burridge, K., Turner, C. E. and Romer, L. H. (1992) Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. J. Cell Biol. **119**, 893–903
7 Cary, L. A. and Guan, J. L. (1999) Focal adhesion kinase in integrin-mediated signaling. Front. Biosci. **4**, D102–D113
8 Zachary, I. and Rozengurt, E. (1992) Focal adhesion kinase (p125^{FAK}): a point of convergence in the action of neuropeptides, integrins, and oncogenes. Cell (Cambridge, Mass.) **71**, 891–894
9 Rankin, S. and Rozengurt, E. (1994) Platelet-derived growth factor modulation of focal adhesion kinase (p125^{FAK}) and paxillin tyrosine phosphorylation in Swiss 3T3 cells. Bell-shaped dose response and cross-talk with bombesin. J. Biol. Chem. **269**, 704–710
10 Matsumoto, K., Nakamura, T. and Kramer, R. H. (1994) Hepatocyte growth factor/scatter factor induces tyrosine phosphorylation of focal adhesion kinase (p125^{FAK}) and promotes migration and invasion by oral squamous cell carcinoma cells. J. Biol. Chem. **269**, 31807–31813
11 Beviglia, L. and Kramer, R. H. (1999) HGF induces FAK activation and integrin-mediated adhesion in MTLn3 breast carcinoma cells. Int. J. Cancer **83**, 640–649
12 Chen, H. C. and Guan, J. L. (1994) Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. Proc. Natl. Acad. Sci. U.S.A. **91**, 10148–10152
13 Polte, T. R. and Hanks, S. K. (1995) Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate p130^{Cas}. Proc. Natl. Acad. Sci. U.S.A. **92**, 10678–10682
14 Harte, M. T., Hildebrand, J. D., Burnham, M. R., Bouton, A. H. and Parsons, J. T. (1996) p130^{cas}, a substrate associated with v-Src and v-Crk, localizes to focal adhesions and binds to focal adhesion kinase. J. Biol. Chem. **271**, 13649–13655
15 Schlaepfer, D. D., Hanks, S. K., Hunter, T. and van der Geer, P. (1994) Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature (London) **372**, 786–791
16 Schlaepfer, D. D. and Hunter, T. (1996) Evidence for *in vivo* phosphorylation of the Grb2 SH2-domain binding site on focal adhesion kinase by Src-family protein-tyrosine kinases Mol. Cell. Biol. **16**, 5623–5633
17 Weiner, T. M., Liu, E. T., Craven, R. J. and Cance, W. G. (1994) Expression of growth factor receptors, the focal adhesion kinase, and other tyrosine kinases in human soft tissue tumors. Ann. Surg. Oncol. **1**, 18–27
18 Maung, K., Easty, D. J., Hill, S. P. and Bennett, D. C. (1999) Requirement for focal adhesion kinase in tumor cell adhesion. Oncogene **18**, 6824–6828
19 Agochiya, M., Brunton, V. G., Owens, D. W., Parkinson, E. K., Paraskeva, C., Keith, W. N. and Frame, M. C. (1999) Increased dosage and amplification of the focal adhesion kinase gene in human cancer cells. Oncogene **18**, 5646–5653
20 Xu, L. H., Yang, X., Craven, R. J. and Cance, W. G. (1998) The COOH-terminal domain of the focal adhesion kinase induces loss of adhesion and cell death in human tumor cells. Cell. Growth. Differ. **9**, 999–1005
21 Frisch, S. M., Vuori, K., Ruoslahti, E. and Chan-Hui, P. Y. (1996) Control of adhesion-dependent cell survival by focal adhesion kinase. J. Cell Biol. **134**, 793–799
22 Hungerford, J. E., Compton, M. T., Matter, M. L., Hoffstrom, B. G. and Otey, C. A. (1996) Inhibition of pp125^{FAK} in cultured fibroblasts results in apoptosis. J. Cell Biol. **135**, 1383–1390
23 Ilic, D., Almeida, E. A., Schlaepfer, D. D., Dazin, P., Aizawa, S. and Damsky, C. H. (1998) Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. J. Cell Biol. **143**, 547–560
24 Illc, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M. and Yamamoto, T. (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature (London) **377**, 539–544
25 Cary, L. A., Chang, J. F. and Guan, J. L. (1996) Stimulation of cell migration by overexpression of focal adhesion kinase and its association with Src and Fyn. J. Cell Sci. **109**, 1787–1794
26 Nolan, K., Lacoste, J. and Parsons, J. T. (1999) Regulated expression of focal adhesion kinase-related nonkinase, the autonomously expressed C-terminal domain of focal adhesion kinase. Mol. Cell. Biol. **19**, 6120–6129
27 Schaller, M. D., Borgman, C. A. and Parsons, J. T. (1993) Autonomous expression of a noncatalytic domain of the focal adhesion-associated protein tyrosine kinase pp125^{FAK}. Mol. Cell. Biol. **13**, 785–791
28 Hildebrand, J. D., Schaller, M. D. and Parsons, J. T. (1993) Identification of sequences required for the efficient localization of the focal adhesion kinase, pp125^{FAK}, to cellular focal adhesions. J. Cell Biol. **123**, 993–1005
29 Richardson, A. and Parsons, T. (1996) A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125^{FAK}. Nature (London) **380**, 538–540
30 Xu, L.-h., Yang, X.-h., Bradham, C. A., Brenner, D. A., Baldwin, A. S., Craven, R. J. and Cance, W. G. (2000) The focal adhesion kinase suppresses transformation-associated, anchorage-independent apoptosis in human breast cancer cells. J. Biol. Chem. **275**, 30597–30604
31 Golubovskaya, V., Beviglia, L., Xu, L. H., Earp, III, H. S., Craven, R. and Cance, W. (2002) Dual inhibition of focal adhesion kinase and epidermal growth factor receptor pathways cooperatively induces death receptor-mediated apoptosis in human breast cancer cells. J. Biol. Chem. **277**, 38978–38987
32 Schaller, M. D., Otey, C. A., Hildebrand, J. D. and Parsons, J. T. (1995) Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. J. Cell. Biol. **130**, 1181–1187

33 Horwitz, A., Duggan, K., Buck, C., Beckerle, M. C. and Burridge, K. (1986) Interaction of plasma membrane fibronectin receptor with talin – a transmembrane linkage. Nature (London) **320**, 531–533

34 Schaller, M. D., Hildebrand, J. D., Shannon, J. D., Fox, J. W., Vines, R. R. and Parsons, J. T. (1994) Autophosphorylation of the focal adhesion kinase, pp125^{FAK}, directs SH2-dependent binding of pp60^{src}. Mol. Cell. Biol. **14**, 1680–1688

35 Chen, H. C., Appeddu, P. A., Isoda, H. and Guan, J. L. (1996) Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase. J. Biol. Chem. **271**, 26329–26334

36 Thomas, J. W., Ellis, B., Boerner, R. J., Knight, W. B., White, II, G. C. and Schaller, M. D. (1998) SH2- and SH3-mediated interactions between focal adhesion kinase and Src. J. Biol. Chem. **273**, 577–583

37 Han, D. C. and Guan, J. L. (1999) Association of focal adhesion kinase with Grb7 and its role in cell migration. J. Biol. Chem. **274**, 24425–24430

38 Eide, B. L., Turck, C. W. and Escobedo, J. A. (1995) Identification of Tyr-397 as the primary site of tyrosine phosphorylation and pp60^{src} association in the focal adhesion kinase, pp125^{FAK}. Mol. Cell. Biol. **15**, 2819–2827

39 Jones, G., and Machado, Jr, J. and Merlo, A. (2001) Loss of focal adhesion kinase (FAK) inhibits epidermal growth factor receptor-dependent migration and induces aggregation of NH_2-terminal FAK in the nuclei of apoptotic glioblastoma cells. Cancer Res **61**, 4978–4981

40 Lobo, M. and Zachary, I. (2000) Nuclear localization and apoptotic regulation of an N-terminal domain focal adhesion kinase fragment in endothelial cells. Biochem. Biophys. Res. Commun. **276**, 1068–1074

41 Stewart, A., Ham, C. and Zachary, I. (2002) The focal adhesion kinase N-terminal domain localises to nuclei and intercellular junctions in HEK 293 and MDCK cells independently of tyrosine 397 and the C-terminal domain. Biochem. Biophys. Res. Commun. **299**, 62–73

42 Xu, L. H., Owens, L. V., Sturge, G. C., Yang, X., Liu, E. T., Craven, R. J. and Cance, W. G. (1996) Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. Cell. Growth Differ **7**, 413–418

43 Sonoda, Y., Kasahara, T., Yokota-Aizu, E., Ueno, M. and Watanabe, S. (1997) A suppressive role of p125^{FAK} protein tyrosine kinase in hydrogen peroxide-induced apoptosis of T98G cells. Biochem. Biophys. Res. Commun. **241**, 769–774

44 van De Water, B., Houtepen, F., Huigsloot, M. and Tijdens, I. B. (2001) Suppression of chemically induced apoptosis but not necrosis of renal proximal tubular epithelial (LLC-PK1) cells by focal adhesion kinase (FAK). Role of FAK in maintaining focal adhesion organization after acute renal cell injury. J. Biol. Chem. **276**, 36183–36193

45 Gilmore, A. P. and Romer, L. H. (1996) Inhibition of focal adhesion kinase (FAK) signaling in focal adhesions decreases cell motility and proliferation. Mol. Biol. Cell **7**, 1209–1224

46 Frisch, S. M. and Francis, H. (1994) Disruption of epithelial cell–matrix interactions induces apoptosis. J. Cell Biol. **124**, 619–626

47 Richardson, A., Malik, R. K., Hildebrand, J. D. and Parsons, J. T. (1997) Inhibition of cell spreading by expression of the C-terminal domain of focal adhesion kinase (FAK) is rescued by coexpression of Src or catalytically inactive FAK: a role for paxillin tyrosine phosphorylation. Mol. Cell. Biol. **17**, 6906–6914

48 Zhang, X., Chattopadhyay, A., Ji, Q. S., Owen, J. D., Ruest, P. J., Carpenter, G. and Hanks, S. K. (1999) Focal adhesion kinase promotes phospholipase C-γ1 activity. Proc. Natl. Acad. Sci. U.S.A. **96**, 9021–9026

49 Sieg, D. J., Hauck, C. R. and Schlaepfer, D. D. (1999) Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J. Cell Sci. **112**, 2677–2691

50 Dunty, J. M. and Schaller, M. D. (2002) The N termini of focal adhesion kinase family members regulate substrate phosphorylation, localization, and cell morphology. J. Biol. Chem. **277**, 45644–45654

51 Martin, S. S. and Leder, P. (2001) Human MCF10A mammary epithelial cells undergo apoptosis following actin depolymerization that is independent of attachment and rescued by Bcl-2. Mol. Cell. Biol. **21**, 6529–6536

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