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Nuclear import of N-terminal FAK by activation of the FcεRI receptor in RBL-2H3 cells

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Abstract

As FAK integrates membrane receptor signalling, yet is also found in the nucleus, we investigated whether nuclear FAK is regulated by membrane receptor activation. Activation of the mast cell FcεRI receptor leads to the release and synthesis of inflammatory mediators as well as increased proliferation and survival. Using RBL-2H3 basophilic leukaemia cells, FAK and the FcεRI receptor were co-localised following cross-linking of IgE with antigen. This also resulted in a significant increase in the nucleus of several N-terminal FAK fragments, the largest of which included the kinase domain but not the focal adhesion targeting domain. This was confirmed using cells that stably expressed recombinant EGFP-FAK. Furthermore, treatment of EGFP-FAK expressing cells with Leptomycin B, an inhibitor of nuclear export, resulted in increased nuclear localisation of EGFP-FAK. Therefore, FAK can shuttle between the nuclear and cytoplasmic compartments and the cellular distribution of N-terminal FAK is regulated by membrane receptor activation.

Keywords: FAK; Focal adhesion kinase; FcεRI; IgE; RBL-2H3; Nuclear import

The focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that plays a key role in the integration of signals from activated membrane receptors, in particular the extracellular matrix (ECM)-activated integrin receptors and tyrosine kinases such as the epidermal growth factor receptor (EGFR) [1,2]. As such, FAK is involved in a diverse range of cellular functions, including cell migration [3], cell survival [4], and gene transcription [5]. The emergence of FAK as a central regulator of cellular signalling is highlighted by evidence showing that FAK is over-expressed in highly malignant and invasive cancers [4,6].

Consistent with a role in membrane receptor signalling, FAK is targeted to specific structures at the membrane, called focal adhesions, by a C-terminal focal adhesion targeting (FAT) domain [7]. Expression of the C-terminal FAT domain alone is an efficient dominant-negative inhibitor of FAK [4,8]. Although much is known about the C-terminal domain of FAK, the role of the N-terminal domain of FAK has only recently been addressed. The N-terminal domain has distant homology with the FERM (band four.1, ezrin, radixin, and moesin) family of proteins that are typically involved in protein–protein interactions between membrane proteins and the actin cytoskeleton [9]. The N-terminal domain of FAK interacts with the Etk kinase [10], ezrin [11], and possibly the EGFR [1].

A more surprising finding has been the demonstration that a truncated N-terminal FAK fragment is present in the nucleus of several different cell lines. The appearance of N-terminal FAK is promoted under pro-apoptotic conditions [12] and aggregates of N-terminal FAK are detected in the nuclei of apoptotic cells [2], while over-expression of an N-terminal FAK fragment induces apoptosis in breast cancer cells [13]. Thus, current evidence would suggest a link between nuclear FAK and cell viability. Although the FAT domain and a larger C-terminal FRNK domain are generated by caspase-dependent cleavage of FAK [14], FAK is also cleaved by calpain in non-apoptotic events such as focal adhesion turnover [15]. The close functional relationship between

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FAK and membrane receptors led us to investigate whether the nuclear localisation of FAK might also be an important signal transduction pathway linked to membrane receptor activation.

Mast cells are key effector cells in allergic diseases such as asthma, releasing a potent cocktail of inflammatory mediators including histamine, proteases, and interleukin (IL)-4 [16]. The RBL-2H3 cell line has been used extensively to study the activation of the mast cell FcεRI receptor by IgE and antigen. Earlier studies have already shown that FAK enhances the FcεRI-dependent release of pre-formed inflammatory mediators [17] and that the N-terminal domain of FAK appears sufficient to do this [18].

In this report we show that stimulation of RBL-2H3 cells with IgE and antigen activates the FcεRI receptor as well as FAK, leading to the accumulation of N-terminal FAK in the nucleus. These results show that the nuclear localisation of FAK can be regulated by normal cellular activity involving membrane receptor activation.

Methods and materials

Cell culture and stimulation of RBL-2H3 cells. Culturing and stimulation of RBL-2H3 cells were done as described [19]. Briefly, RBL-2H3 cells were maintained in RPMI 1640 (Gibco, Invitrogen) with 10% FBS. One day prior to stimulation, cells were passaged and incubated overnight with 10 μg/ml of monoclonal anti-dinitrophenyl (DNP) IgE (Clone SPE-7, Sigma). Cells were then washed in PBS and stimulated by adding the indicated amount of antigen (DNP-Albumin conjugate, Calbiochem–Novabiochem) in SG buffer (119 mM NaCl, 5 mM KCl, 25 mM Pipes, 5.6 mM dextrose, and 0.4 mM $MgCl_2$, pH 7) supplemented with 1 mM $CaCl_2$, 1% BSA, and 2 mM glutamine for up to 2 h. Controls were treated with supplemented SG buffer but in the absence of antigen for the same amount of time. Degranulation was determined by the release into the culture supernatent of β-hexosaminidase using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (PNAG, Sigma). Total cellular β-hexosaminidase was determined by lysing non-treated control cells in SG buffer with 0.1% Triton X-100.

Confocal microscopy. Each experiment used 3×10^5 cells plated on glass coverslips in six-well plates. Following stimulation, cells were washed in ice-cold PBS and fixed for 5 min with 4% paraformaldehyde (PFA). Cells were washed three times in phosphate-buffered saline (PBS)/0.01% Triton X-100 and then blocked for 30 min in a buffer containing 5% horse serum, 1% BSA, and 0.1% Triton X-100 in 1× PBS. Combinations of polyclonal anti-FAK (A-17, Santa-Cruz Biotechnology) with monoclonal anti-FcεRI β-subunit (a gift from Dr. Juan Rivera, NIAMS/NIH) or monoclonal anti-FAK (Clone 4.47, Upstate Biotechnology), with polyclonal anti-p107 (C-18, Santa-Cruz Biotechnology) were each diluted 1:100 in the same buffer and added to the cells for 2 h at room temperature. Anti-FAK antibodies A-17 and 4.47 both recognised epitopes in the N-terminal domain of FAK. The recombinant myc epitope was detected using a monoclonal antibody against residues 409–420 (Clone 9E10, Upstate Biotechnology). Cells were washed three times in PBS/0.01% Triton X-100 and then incubated with 1:250 dilutions of goat anti-mouse Alexa-489 and goat anti-rabbit Alexa-568 (Molecular Probes) for 1 h at room temperature. Cells were washed extensively in PBS/0.01% Triton X-100 and analysed using an Optiscan confocal microscope at 600× magnification.

Nuclear and cytoplasmic extracts. 3×10^6 cells were plated onto 10 cm plates and stimulated as indicated. Cells were then placed on ice and harvested into ice-cold PBS by scraping. Cells were centrifuged at 1500*g* for 5 min at 4 °C, resuspended in 100 μl of 10 mM Hepes (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 0.2% NP-40, 1 mM DTT, and incubated on ice for 15 min. Lysed cells were centrifuged at 1500*g* for 5 min at 4 °C, the cytoplasmic lysate was removed, and pelleted nuclei were washed once in 500 μl of the same buffer, but without NP-40. Nuclear proteins were extracted by incubating nuclei in 50 μl of 20 mM Hepes (pH 7.9), 420 mM NaCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 25% glycerol, and 1 mM DTT on ice for 30 min with vigorous vortexing every 10 min. All buffers included protease and phosphatase inhibitor cocktails (Sigma) at 10× the recommended concentrations. Nuclear and cytoplasmic lysates were centrifuged for 30 min at 20,000*g* at 4 °C, protein concentration of the cleared lysates was determined, and an equal volume of 2× SDS loading buffer was added to each sample. The purity of each fraction was determined in each case by checking for the distribution of cytoplasmic (β-subunit of the FcεRI receptor) and nuclear (p107) proteins.

Immuno-precipitation and Western blotting. For immuno-precipitation, 3×10^6 cells were plated onto a 10 cm plate and stimulated as indicated. Cells were then placed on ice and harvested in ice-cold PBS by scraping. Cells were centrifuged at 1500*g* for 5 min at 4 °C and then lysed for 15 min on ice in 1 ml lysis buffer (50 mM Hepes, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 10% glycerol, pH 7.2) with added protease and phosphatase inhibitor cocktails (Sigma) at 10× the recommended concentrations. The lysate was cleared by centrifugation at 20,000*g* for 30 min at 4 °C. The lysate was divided into two 500 μl aliquots and 1 μl of either Anti-FAK (Clone 4.47) or anti-β subunit FcεRI antibodies were added for 2 h with rotation at 4 °C. Protein G–Sepharose (Amersham) was then added for a further 2 h, after which the lysates were centrifuged at 700*g* for 1 min and washed three times in lysis buffer. For Western blotting of fractionated cell extracts or immuno-precipitated proteins, proteins were resolved on either 7.5% or 10% gels, transferred to nitrocellulose, and blocked in PBST (PBS with 0.1% Tween 20)/5% skim milk. Either anti-FAK (A-17), anti-β-subunit, anti-phosphotyrosine (clone 4G10, Upstate Biotechnology), anti-p107, mouse anti-GFP (Roche Diagnostics) or mouse monoclonal anti-SUMO (Zymed) was added at a dilution of 1:2000 and incubated overnight at 4 °C with gentle shaking. Membranes were washed extensively in PBST and secondary anti-mouse or anti-rabbit HRP-conjugated antibodies were added in PBST/5% milk at a dilution of 1:20,000 for 1 h at room temperature. Membranes were stripped by washing for 1 h in 0.1 M glycine (pH 2.5). Proteins were visualised using the SuperSignal West Pico chemiluminescent reagent (Pierce).

Construction of pEGFP-FAK and transfection of RBL-2H3 and NIH3T3 cells. Full-length FAK was amplified from a human fetal brain cDNA library by PCR and cloned into pEGFP-C1 (Clontech). RBL-2H3 cells were stably transfected by mixing 6×10^6 cells with 20 μg plasmid in 800 μl serum-free RPMI media and then electroporated at 310 V and 950 μF using a Bio-Rad Gene Pulser. Cells were selected in 300 μg/ml G418 and single colonies were selected for analysis. Mouse NIH3T3 cells were maintained in DMEM supplemented with 10% FCS and passaged one day prior to transfection. Cells were transfected using GenePORTER2 (Gene Therapy Systems) as previously described [2]. The constructs pFATmycZeo and $pEGFP\Delta NFAK_{361}$ have also been described previously [2].

Results

FAK is activated by IgE and antigen

Using the rat RBL-2H3 basophilic leukemia line, we first examined whether FAK is activated by cross-linking of the FcεRI receptor by IgE and specific antigen. To do this, cells were incubated overnight with anti-

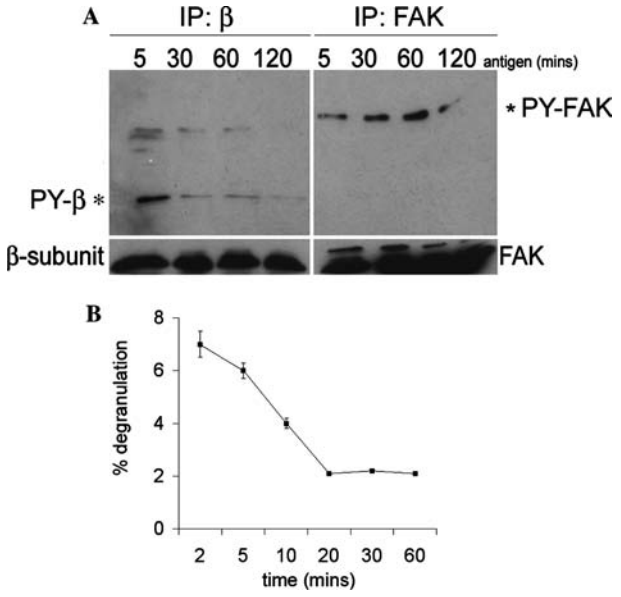

Fig. 1. (A) Phosphorylation of the β-subunit of the FcεRI receptor and FAK. RBL-2H3 cells were stimulated for the indicated times with 10 ng/ml antigen before being analysed by immuno-precipitation (IP) using antibodies recognising either the β-subunit of the FcεRI receptor (β) or FAK. Phosphorylated β-subunit (PY-β) and FAK (PY-FAK) are indicated. (B) Degranulation of RBL-2H3 cells. % degranulation is defined as the release of β-hexosaminidase per minute as a percent of total cellular β-hexosaminidase.

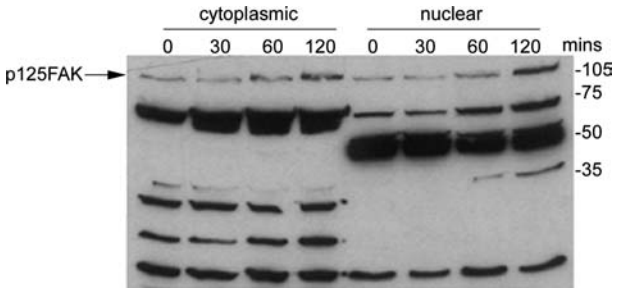

Fig. 2. Time-dependent import of an N-terminal FAK fragment in the nucleus of stimulated RBL-2H3 cells. Cytoplasmic and nuclear lysates were prepared from RBL-2H3 cells stimulated with 10 ng/ml antigen for up to 120 min. FAK was visualised using an antibody that recognises the N-terminal domain (A-17). Full-length 125 kDa FAK is indicated (p120FAK).

DNP IgE and then stimulated for between 5 and 120 min with antigen (DNP-BSA). Whole-cell lysates were prepared and phosphorylation of FAK and that of the β-subunit of the FcεRI receptor were compared using an immuno-precipitation assay. Maximal tyrosine phosphorylation of the β-subunit of the FcεRI receptor was extremely rapid, occurring within 5 min (Fig. 1A, IP: β) whereas FAK tyrosine phosphorylation continued to increase for up to 60 min after the addition of antigen (Fig. 1A, IP: FAK). Although FAK is required for maximal degranulation [17], comparison of these results with the release of β-hexosaminidase, a marker for degranulation, showed the rapid activation of the FcεRI receptor parallels the release of β-hexosaminidase, which reached a maximum after 2 min of incubation with antigen (Fig. 1B).

N-terminal FAK is imported into the nucleus of stimulated RBL-2H3 cells

The nuclear localisation of an N-terminal fragment of FAK in several different cell lines [2,12] suggests FAK might participate in a novel signalling pathway between the cell membrane and the nucleus. To test whether activation of the FcεRI receptor might also result in the nuclear accumulation of FAK, RBL-2H3 cells were stimulated for up to 120 min with 10 ng/ml of specific antigen (DNP-BSA) and nuclear and cytoplasmic fractions were prepared. Using a polyclonal antibody that recognised the N-terminal domain of FAK (A-17), 125 kDa FAK was detected in the cytoplasmic fractions (Fig. 2, cytoplasmic). Several lower molecular weight FAK fragments were also detected under all conditions and most probably arose due to the high level of endogenous proteolytic activity in this cell line (data not shown). Several N-terminal FAK fragments were also detected in the nucleus, although the molecular weight of these fragments differed from those seen in the cytoplasmic fraction. The largest nuclear FAK fragment was approximately 105 kDa in size, with three other FAK fragments of approximately 75, 50, and 40 kDa also visible. As the polyclonal anti-FAK antibody recognises an epitope within the first 65 amino acids of the N-terminal domain, these results suggested C-terminal proteolysis of FAK resulted in nuclear import.

To confirm that import of FAK into the nucleus of stimulated RBL-2H3 cells was in response to FcεRI activation, and not as a result of non-specific proteolysis during the extraction procedure, we used monoclonal anti-FAK (clone 4.47) and polyclonal anti-p107 antibodies with confocal microscopy to quantify the number of nuclei showing visible FAK immuno-reactivity. A comparison of cells incubated with IgE in either the absence or presence of antigen (Fig. 3A; −antigen, +antigen, respectively) revealed a significantly greater number of nuclei showed detectable FAK immuno-reactivity in the presence of antigen (Fig. 3B, 0.34 ± 0.1 vs. 0.60 ± 0.08, $p < 0.025$, mean ± SEM). Consistent with this, cytoplasmic staining of FAK was more prominent in cells not stimulated with antigen (Fig. 3A, arrowhead, middle panel). Significantly, incubation of RBL-2H3 cells with IgE alone was also sufficient to induce a marked concentration of FAK at the cell membrane along points of cell–cell contact (Fig. 3A, arrowhead, top panel). To test whether such aggregates of FAK also co-localised with the FcεRI receptor, RBL-2H3 cells were incubated overnight with IgE, stimulated for

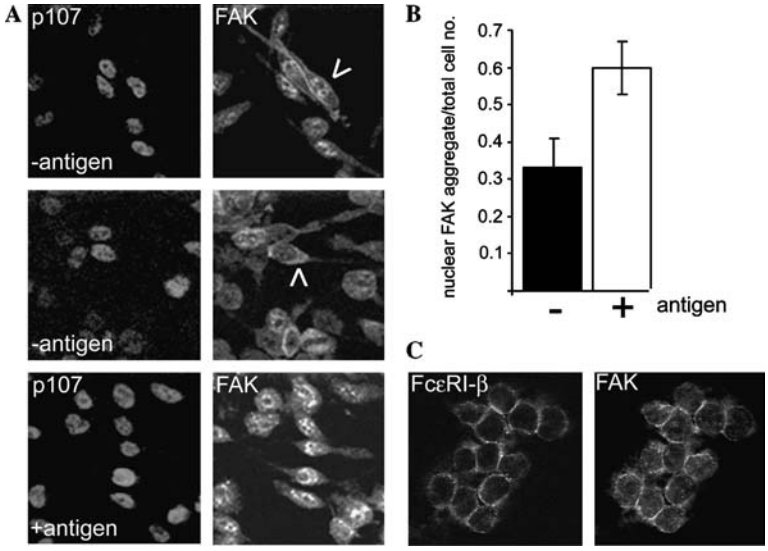

Fig. 3. (A) Cellular distribution of FAK in cells incubated overnight with anti-DNP IgE and then for 120 min in either the absence (−antigen) or presence (+antigen) of 10 ng/ml antigen (DNP-albumin). Arrowheads indicate concentration of FAK at points of cell–cell contact and cytoplasmic localisation. FAK was visualised using a monoclonal antibody (4.47) that recognised an epitope in the N-terminal domain and nuclei were identified using a polyclonal anti-p107 antibody. (B) Quantification of the number of cells with visible FAK nuclear localisation in the absence (black bar) or presence (white bar) of antigen. The analysis was performed by randomly selecting fields and counting the total number of cells (−antigen, $n = 131$; +antigen, $n = 141$) and the number of cells that exhibited nuclear FAK immuno-reactivity. Results are means ± SEM and were analysed using the Mann–Whitney test. (C) Co-localisation of the FcεRI receptor and FAK along regions of cell–cell contact on stimulated RBL-2H3 cells. Cells were incubated overnight with anti-DNP IgE and stimulated for 60 min with 10 ng/ml of antigen. Localisation of the FcεRI receptor and FAK was visualised using monoclonal anti-FcεRI β-subunit and polyclonal FAK (A-17) antibodies.

60 min with antigen, and then examined by confocal microscopy using the monoclonal anti-β-subunit and polyclonal A-17 anti-FAK antibodies. The results showed that both FAK and the FcεRI were highly localised along regions of cell–cell contact (Fig. 3C).

A cleaved N-terminal EGFP-FAK fragment is imported into the nucleus

Using polyclonal (A-17) and monoclonal (4.47) anti-FAK antibodies that recognised epitopes within the N-terminal domain, we have shown that the cellular distribution of FAK in RBL-2H3 cells was altered by incubation with IgE and antigen (Figs. 2 and 3). To confirm that the key determinant of nuclear localisation resides in the N-terminal domain, we transfected NIH3T3 cells with plasmids expressing full-length and truncated FAK proteins tagged at either the N-terminal end with EGFP (Fig. 4; FAK, N-FAK$_{361}$) or at the C-terminal end with the myc epitope (Fig. 4; C-FAK$_{FAT}$). Expression of full-length FAK was detected throughout the cell including focal adhesion-like structures (Fig. 4, left panel, FAK). An N-terminal 361 amino-acid fragment was effectively targeted to the nucleus, but cytoplasmic localisation and targeting to the cell membrane was also observed (Fig. 4, middle panel, N-FAK$_{361}$). In contrast, the C-terminal FAT domain was restricted to the cytoplasm and also showed strong localisation to focal adhesion-like structures (Fig. 4, right panel, C-FAK$_{FAT}$). Therefore, in agreement with results from stimulated RBL-2H3 cells, the N-terminal domain of FAK is the primary determinant of nuclear localisation. Furthermore, the relative distribution of FAK and N-FAK$_{361}$ between the nucleus and the cytoplasm would suggest that loss of the C-terminal domain was a pre-requisite for effective nuclear import (Fig. 4, FAK, N-FAK$_{361}$).

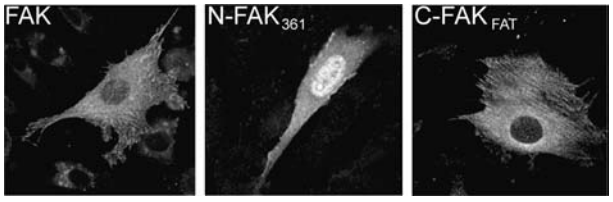

Fig. 4. The N-terminal domain targets FAK to the nucleus. To confirm that the N-terminal domain of FAK is required for nuclear import, NIH3T3 cells were transfected with plasmids that expressed either EGFP-tagged full-length FAK (left panel, FAK), EGFP-tagged N-terminal FAK (middle panel, N-FAK$_{361}$) or a myc-tagged C-terminal FAT domain (right panel, C-FAK$_{FAT}$).

To confirm that nuclear FAK in stimulated RBL-2H3 cells arose through the cleavage and loss of C-terminal sequences, RBL-2H3 cell lines that stably expressed N-terminal-tagged EGFP-FAK were produced. Two transfected cell lines were selected (RBL/EGFP-FAK$_1$ and RBL/EGFP-FAK$_{12}$), with the highest level of expression seen in RBL/EGFP-FAK$_{12}$. When

compared to non-transfected RBL-2H3 cells, activation with IgE and antigen resulted in a 20% and 5% increase in β-hexosaminidase release in RBL/EGFP-FAK$_{12}$ and RBL/EGFP-FAK$_1$, respectively (data not shown). This is in agreement with previous results showing that FAK enhances FcεRI-dependent degranulation [17]. All further analyses were done using RBL/EGFP-FAK$_{12}$ cells.

The RBL/EGFP-FAK$_{12}$ cell line was incubated overnight with IgE and either not stimulated with antigen (Fig. 5A, 0 min antigen) or stimulated with antigen for 30 or 120 min (Fig. 5A). This resulted in the time-dependent increase in the nucleus of FAK and EGFP immuno-reactive fragments of approximately 130 and 105 kDa (Fig. 5A, arrows). Since the EGFP tag was at the N-terminal end of FAK and as the molecular weight of full-length EGFP-FAK was 155 kDa (125 kDa FAK plus 30 kDa EGFP), the 105 and 130 kDa fragments would correspond to C-terminal deletions of approximately 20 and 45 kDa. This is consistent with proteolysis of the C-terminal FAT and FRNK [14] domains that map to amino acids 904/1052 and 668/1052, respectively.

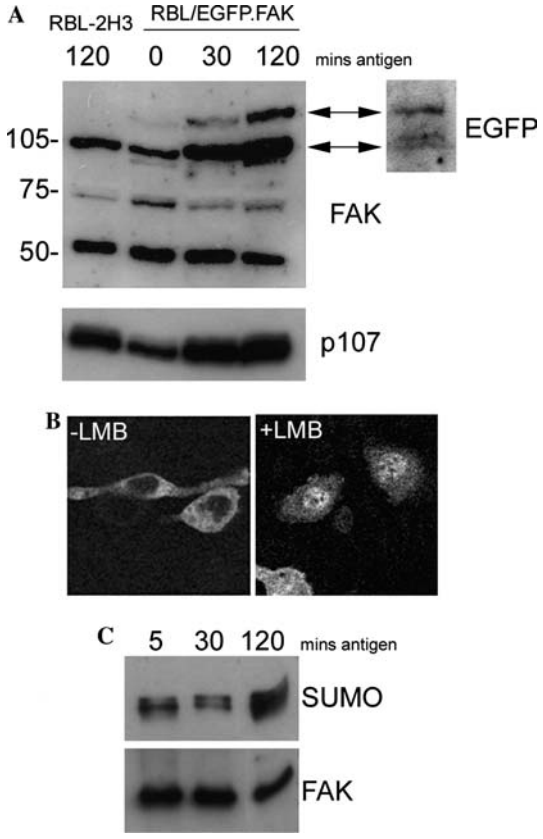

Fig. 5. (A) N-terminal EGFP-FAK is imported into the nucleus of activated RBL/EGFP-FAK cells. Shown are nuclear lysates from RBL-2H3 or RBL/EGFP-FAK$_{12}$ cells stimulated with 10 ng/ml antigen for the indicated times. FAK and EGFP-FAK were visualised using anti-FAK (FAK) and anti-EGFP (EGFP) antibodies. EGFP-FAK is indicated by arrows. The nuclear protein p107 is also shown. (B) EGFP-FAK is retained in the nucleus of non-stimulated cells in the presence of leptomycin B. Cells were incubated in SG media plus 50 ng/ml leptomycin B for 2 h and analysed by direct fluorescence. (C) FAK is modified by SUMO. RBL-2H3 cells were stimulated with 10 ng/ml antigen for the indicated times and then harvested for an immunoprecipitation assay using the anti-FAK antibody (A-17). The Western blot was probed with an anti-SUMO antibody (SUMO), stripped, and re-probed using and anti-FAK antibody (FAK).

Although effective nuclear import of the largest N-terminal EGFP-FAK fragment was dependent upon antigen stimulation, fragments of 105 kDa and smaller were clearly detected in nuclear lysates from non-stimulated cells (Fig. 5A, 0 vs. 120 min antigen). This was also in agreement with results from non-transfected RBL-2H3 cells (Fig. 2) and led us to investigate whether inhibiting nuclear export in non-stimulated cells might lead to retention of FAK in the nucleus. Incubation of non-stimulated RBL/EGFP-FAK$_{12}$ cells for 2 h in leptomycin B, an inhibitor of nuclear export, resulted in the appearance of EGFP in the cell nuclei (Fig. 5B). Consistent with results from non-stimulated cells (Figs. 2 and 5A), the major nuclear fragment in nuclear lysates from LMB-treated RBL/EGFP-FAK$_{12}$ cells was 105 kDa (data not shown). Therefore, N-terminal FAK can shuttle between the nucleus and the cytoplasm.

A recent report showing FAK is modified by SUMO [20] provides further evidence for the regulated import of FAK into the nucleus. To confirm that FAK is also modified by SUMO in RBL-2H3 cells, cells were stimulated for between 5 and 120 min, lysates were immunoprecipitated using an anti-FAK antibody (4.47) and then probed using an anti-SUMO antibody. This showed that SUMO modification of FAK was visible after 5 min of stimulation and increased after 120 min of stimulation (Fig. 5C). Taken together, these results showed that an N-terminal domain of FAK, arising from cleavage of the FAT domain, was imported into the nucleus following FcεRI activation.

Discussion

The activation of membrane receptors by growth factors, cytokines, and the ECM initiates a signal transduction cascade from the cell membrane to the nucleus. It is well accepted that FAK has a key role in integrating signals from growth factor and integrin receptors at the cell membrane [1] as well as propagating signals to other intracellular kinases [21]. We have now shown that the activation of FAK by the FcεRI receptor also results in the accumulation of an N-terminal FAK fragment in the nucleus of RBL-2H3 cells. Retention of EGFP-FAK in non-stimulated cells in the presence of leptomycin B showed that FAK was not only restricted to the cell membrane and cytoplasm but is also imported and exported from the cell nucleus.

We detected FAK in the cytoplasm and along the membrane of RBL-2H3 cells incubated with IgE in the absence of antigen (Fig. 3A), while in the presence of

antigen there was a marked co-localisation of FAK and the FcεRI receptor at cell–cell junctions (Fig. 3C). The co-localisation of FAK and the FcεRI receptor is in agreement with biochemical studies showing that FAK enhances FcεRI-dependent mast cell degranulation [17] and the localisation of FAK at cell–cell junctions is similar to that reported in several other cell types [22]. Therefore, since FAK is a multi-domain protein able to participate in a range of protein–protein interactions, FAK may serve as a type of molecular scaffold for the FcεRI receptor. However, the difference in the kinetics of the phosphorylation of FAK and the FcεRI receptor (Fig. 1) suggested that activation of the FcεRI receptor might be an essential pre-requisite for the activation of FAK and other signalling proteins at the mast cell membrane.

Data presented here and in other cell lines [2,12] showed the distribution of FAK between the membrane and the nucleus was dictated by the presence or absence of the C-terminal FAT domain, yet the question remains as to where the biologically relevant sequences reside within the N-terminal sequences of FAK. Expression of a 423 amino acid (approximately 47 kDa) N-terminal FAK fragment induced apoptosis in breast cancer cell lines independently of the Tyr-397 auto-phosphorylation site [13], which is otherwise essential for FAK activation at focal adhesions [7]. In human brain tumour cells a 361 amino acid (approximately 40 kDa) N-terminal fragment did not induce apoptosis, but instead formed nuclear aggregates in apoptotic cells [2]. In HEK293 or epithelial MDCK cells, a 386 amino acid N-terminal FAK fragment was localised both in the nucleus and at intercellular junctions but had no obvious biological effect [22]. Therefore, the N-terminal domain of FAK is targeted to both the membrane and the nucleus independently of Tyr-397. However, since the nuclei of RBL-2H3 cells contained an approximately 105 kDa N-terminal fragment that included both Tyr-397 and the kinase domain, these sequences may be required for the full biological activity of the N-terminal domain of FAK. Given that the distribution of N-terminal FAK is sensitive to cell viability [2,12] and that binding of IgE to the FcεRI receptor is a survival signal [23], one role might be in cell survival and proliferation.

In addition to FAK, at least two other focal adhesion proteins, Hic-5 [24] and Zyxin [25], are also imported into the nucleus. The nuclear role of Zyxin is not known but Hic-5 acts as a transcriptional co-activator to increase transcription of the *c-fos* gene [24]. The membrane receptor tyrosine kinases EGFR [26] and ErbB-4 [27] also enter the nucleus where the EGFR acts as a transcriptional co-activator [26]. The interaction of the N-terminal domain of FAK with proteins such as the Etk kinase, ezrin, and PIAS1 [20], together with the observation reported here that FAK shuttles between the nucleus and cytoplasm, suggests that one role for FAK might be as a carrier protein for other proteins into the nucleus. This idea is consistent with the modification of the N-terminal domain of FAK at Lys-152 by SUMO [20].

The emergence of FAK as a signalling protein at both the cell membrane and in the nucleus has important implications for the transfer of information from cell surface receptors to the nucleus. It is now important to clearly define the role of FAK in the nucleus and understand how this contributes to the activity of specific cell types, particularly in mast cells in the context of allergic disorders.

Acknowledgments

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