Analysis of FAK-associated signaling pathways in the regulation of cell cycle progression

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Abstract Focal adhesion kinase (FAK) is an important mediator of signal transduction pathways initiated by integrins in cell migration, survival and cell cycle regulation. The ability of FAK to mediate integrin signaling in the regulation of cell cycle progression depends on the phosphorylation of Tyr397, which implies a functional significance for the formation of FAK signaling complexes with Src, phosphatidylinositol-3-kinase (PI3K) and Grb7. We have previously described a FAK mutant, D395A, that selectively disrupts FAK binding to PI3K, but allows FAK association with Src. Using this mutation in a mislocalized FAK mutant background, we show here that formation of a FAK/PI3K complex is not sufficient for cell cycle progression but the formation of a FAK/Src complex plays an essential role. We also show that mutation of D395 to A disrupted FAK association with Grb7. This suggests that a FAK/Grb7 complex is not involved in the cell cycle regulation either, which is supported by direct analysis of cells expressing a dominant negative Grb7 construct. Finally, we provide evidence that the Src-dependent association of FAK with Grb2 and p130^{Cas} are both required for the regulation of cell cycle progression by FAK. Together, these studies identify important FAK downstream signaling pathways in cell cycle regulation.

Key words: Focal adhesion kinase; Signaling complex; Signal transduction; Cell cycle progression; MAP kinase

1. Introduction

Focal adhesion kinase (FAK) is a 125 kDa protein tyrosine kinase that plays a major role in signal transduction mediated by integrins [1,2]. Integrin signaling through FAK has been shown to regulate cell migration [3–5], proliferation [4,6] and cell survival [7]. The ability of FAK to regulate these cellular processes depends upon its localization to focal contacts and its ability to form protein complexes with other signaling molecules [6,8–11].

Y397 is the major phosphorylation site in FAK [12]. Upon phosphorylation, Y397 serves as the binding site for the SH2 domains of other proteins such as Src [13,14], phosphatidylinositol-3-kinase (PI3K) [15], PLC$_{\gamma 1}$ [16] and Grb7 [17]. It has been shown previously that Asp395 in FAK is critical for the association of PI3K with FAK at Y397, but mutation of this residue to Ala has no effect on FAK/Src association [9,11]. Use of this mutant allows the direct examination of FAK/PI3K versus FAK/Src association in different cellular processes [9,11,18]. Binding of Src to FAK at Y397 allows Src to phosphorylate Y925 of FAK, thus creating a binding site for the adapter protein Grb2 [19,20]. Grb2 exists in complex with SOS, and potentially links FAK to the Ras/MAPK cascade [19]. FAK also contains proline-rich motifs capable of binding the SH3 domain of the adapter protein p130^{Cas} (Cas) [21]. Cas is phosphorylated on multiple tyrosine residues by Src [22,23], which forms binding sites for other signaling molecules bearing SH2 domains [24].

Several previous studies have suggested a role for FAK signaling pathways in the regulation of cell cycle progression [4,6,10,25]. Microinjection of a FAK-inhibitory antibody blocks the cell cycle at the G1/S boundary [25]. In addition, microinjection of the C-terminal domain of FAK (i.e. FRNK) causes cell cycle arrest [4]. One study has described a dependence on FAK signaling to JNK through Cas for cell cycle regulation [10]. Finally, our laboratory has shown that a truncated FAK mutant (ΔC14) unable to localize to focal contacts inhibits Erk activation and cell cycle progression through the G1 phase [6]; this effect was dependent on the phosphorylation of Y397 [6]. While these studies clearly demonstrated a role for FAK in the regulation of cell cycle progression, the relative contributions made by distinct FAK signaling complexes (e.g. FAK/PI3K versus FAK/Src) were not examined, and to date poorly understood.

Here we investigate the role of several signaling molecules that bind to FAK at phosphorylated Y397 including PI3K, Src and Grb7. We showed that while FAK/PI3K and FAK/Grb7 complexes are not involved, the FAK/Src complex plays an essential role in the cell cycle regulation by FAK. Furthermore, we showed that FAK interactions with Grb2 and Cas, both downstream events of FAK/Src complex formation, are required for the regulation of cell cycle progression by FAK.

2. Materials and methods

2.1. Antibodies

The monoclonal antibody 12CA5 (α-HA) [26] and KT3 [5] have been described previously. The rabbit polyclonal α-p85 [26] and α-KC (α-FAK) [13] sera have also been described. The following antibodies were purchased as indicated: α-BrdU mouse mAb from Sigma (St. Louis, MO, USA); rabbit α-pErk from New England Biolabs (Beverly, MA, USA), rabbit α-Src, and α-Erk from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Constructs

The constructs pKH3-FAK, pKH3-FAK Y397F [26], pKH3-FAK

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D395A, pKH3-FAK P712/715A [9], pKH3-FAK ΔC14 [6], pDHGST and pDHGST-Grb7 [17] have been described. The constructs pCDM8-FAK, pCDM8-FAK Y397F [8] and pCDM8-FAK D395A [9] have also been previously described. pKH3-FAK ΔC14F was generated by digesting pKH3-FAK Y397F with *Msc*I and *Nhe*I. This 1.8 kb fragment was gel purified and subcloned into pKH3-FAK ΔC14 in which the corresponding 1.8 kb fragment had been excized. Similarly, pKH3-FAK ΔC14A was generated by digesting pKH3-FAK D395A with *Msc*I and *Nhe*I and subcloning this fragment into pKH3-FAK ΔC14 as described above. pKH3-FAK Y925F and pKH3-FAK COMBO were generated by digesting pCDM8-FAK Y925F and pCDM8-FAK COMBO [8] with *Msc*I and *Nhe*I and subcloning the resulting fragments into pKH3-FAK, in which the corresponding fragment had been removed.

2.3. Transient transfections

NIH 3T3 and HEK 293T cells were transfected with the indicated constructs using the LipofectAmine and PLUS™ transfection reagents (Life Technologies) according to the manufacturer's instructions. NIH 3T3 cells were maintained in DME supplemented with 10% CS. These were transfected with pKH3 (C), pKH3-FAK ΔC14, pKH3-FAK ΔC14F, pKH3-FAK ΔC14A, pKH3-FAK Y925F, pKH3-FAK P712/715A and pKH3-FAK COMBO. One day following transfection, cells were processed for the analysis of BrdU incorporation as described below. HEK 293T cells were maintained in DME supplemented with 10% FBS. Cells were co-transfected with pDHGST-Grb7 and pCDM8-FAK, pCDM8-FAK Y397F, and pCDM8-FAK D395A, or pDHGST and pCDM8-FAK. These were then processed for pull-down assays 2 days following transfection.

2.4. Cell lines

pKH3-FAK D395A was digested with *Msc*I and *Nhe*I. This 1.8 kb fragment was subcloned into pTet-Splice FAK ΔC14 [6] in which the corresponding fragment had been excized. Ligation of these fragments produced pTet-Splice-FAK ΔC14A, and was co-transfected with pTet-tTak and pSV2neo as described previously [6]. Likewise, pKH3-FAK Y925F, pKH3-FAK P712/715A and pKH3-FAK COMBO were digested with *Msc*I and *Nhe*I. These 1.8 kb fragments were subcloned into pTet-Splice FAK [6] in which the corresponding fragment had been excized. Ligation of these fragments produced pTet-Splice-FAK Y925F, pTet-Splice-FAK P712/715A and pTet-Splice-FAK COMBO. These were co-transfected with pTet-tTak and pSV2neo as described previously [6]. For all, cells were selected in medium containing 0.5 mg/ml G418 and screened for the inducible expression of FAK ΔC14A and FAK Y925F, FAK P712/715A and FAK COMBO as stated previously [6]. Cell lines capable of the inducible expression of FAK ΔC14, FAK ΔC14F and control cells (MOCK) have been described previously [6]. NIH 3T3 cells capable of the inducible expression of the Grb7 SH2 domain have also been described previously [17]. All cell lines were maintained in DME supplemented with 10% CS, 0.5 mg/ml G418 and 0.4 μg/ml tetracycline to suppress the expression of the FAK constructs until required for the indicated experiments. Induction of exogenous proteins was achieved by culturing cell lines in DME supplemented with 10% CS and 0.5 mg/ml G418 for at least 16 h.

2.5. Cell lysis, immunoprecipitations and immunoblotting

Cells were washed twice with ice-cold phosphate-buffered saline and lyzed with RIPA buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, 1 mM sodium vanadate, 10 mM sodium pyrophosphate, 10 mM NaF, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulfate, 10 μg/ml leupeptin and 1 mM PMSF).

Lysates were cleared by centrifugation and total protein concentrations were determined using the BioRad Protein assay (Hercules, CA, USA). Immunoprecipitations were carried out at 4°C by incubating cell lysates with α-Src or α-p85 followed immediately by incubation with protein G agarose (Sigma). After washing, the beads were resuspended in SDS–PAGE sample buffer, boiled and resolved via SDS–PAGE.

Immunoblots were performed as indicated with α-HA (1:1000), α-pErk (1:1000), α-Erk (1:1000), α-FAK (1:5000) and the KT3 mAb (1:5000) using the enhanced chemiluminescence system as described [13]. In most experiments, equal amounts of WCL were analyzed directly by immunoblotting.

2.6. Pull-down assay

The association of Grb7 with FAK and its mutants was performed as described previously [17]. Briefly, transiently transfected HEK 293T cells were starved in serum-free DME for 12 h, and re-plated on FN-coated (10 μg/ml) dishes in serum-free medium for 30 min and lyzed in RIPA buffer. Lysates were incubated with glutathione agarose, washed and resuspended in sample buffer and prepared for immunoblotting with the indicated antibodies.

2.7. Analysis of 5′-BrdU incorporation

Transiently transfected NIH 3T3 cells were serum starved for 48 h in DME supplemented with 0.5% CS. They were then re-plated on FN-coated (10 μg/ml) glass coverslips and incubated for 16 h in the presence of 100 μM BrdU in DME plus 10% CS. Cells were then processed for immunofluorescence staining with α-BrdU and α-FAK as described [27]. The primary antibodies used were α-FAK (1:300) and α-BrdU (1:300). The secondary antibodies used were rhodamine-conjugated anti-mouse (1:300; Sigma) and FITC-conjugated anti-rabbit (1:300; Sigma). Cellular DNA was digested with 0.5 U/μl DNaseI (New England Biolabs, Beverly, MA, USA) for 30 min at 37°C prior to staining with the primary antibodies. At least 30 positively transiently transfected cells were scored for BrdU incorporation in each independent experiment. For experiments examining DNA synthesis using the Grb7 SH2 inducible cell line, cells were processed as described previously [6]. In all of the experiments listed above, statistical analyses were performed using Minitab Xtra 10.5 software (Minitab, State College, PA, USA).

3. Results

Previous studies from our laboratory have suggested that Y397 of FAK played an essential role in the regulation of cell cycle progression by FAK [6]. PI3K association with receptor tyrosine kinases has been shown to mediate mitogenic effects of growth factor signaling pathways [28]. Therefore, FAK association with PI3K through Y397 might also play a role in the downstream pathways leading to cell cycle regulation. To investigate a potential role for the FAK/PI3K association, we first tested whether a mislocalized FAK mutant (ΔC14), which inhibited cell cycle progression [6], could bind to PI3K as wild type FAK [26]. Lysates were prepared from NIH 3T3 cells inducibly overexpressing ΔC14 or ΔC14F (ΔC14 mutation with Y397F), as well as control cells (Mock). Lysates were then immunoprecipitated with α-p85 or α-Src followed by immunoblotting with α-HA to detect the presence of HA-tagged FAK mutants. Fig. 1 shows that ΔC14 was associated with PI3K as well as Src in these cells. Mutation of Y397 to F

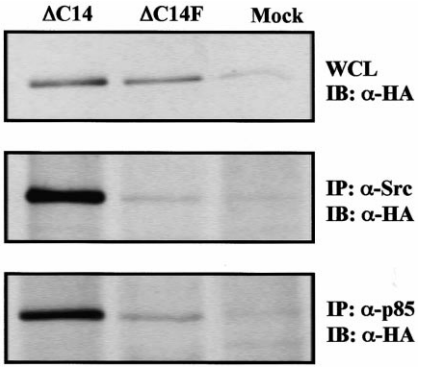

Fig. 1. FAK ΔC14 associates with PI3K. Lysates from NIH 3T3 cells induced to express ΔC14, ΔC14F or control cells (MOCK) were immunoprecipitated with α-Src or α-p85 (regulatory subunit of PI3K). The immunoprecipitates were analyzed by immunoblotting with α-HA (middle and bottom panels). Aliquots of lysates were also analyzed by immunoblotting with α-HA directly (top panel).

abolished its binding to both PI3K and Src. Analysis of the lysates by immunoblotting with α-HA directly confirmed similar expression levels of ΔC14 and ΔC14F in these cells. Together, these results suggested that ΔC14 might inhibit cell cycle progression by competing with the endogenous FAK for binding PI3K and/or Src family kinases.

To determine directly the potential role of a FAK/PI3K complex in the regulation of cell cycle progression by integrins, we created a FAK mutant with D395 mutated to A and lacking the last 14 amino acids (ΔC14A). Mutation of D395 to A selectively disrupts the association of FAK with PI3K, but did not affect FAK binding to Src [9]. Therefore, the mislocalized ΔC14A mutant is expected to inhibit the endogenous FAK/Src complex, but not the FAK/PI3K complex. NIH 3T3 cells were transiently transfected with pKH3-FAK ΔC14A (ΔC14A), pKH3-FAK ΔC14F (ΔC14F; Y397 mutated to F and lacking the last 14 amino acids), or pKH3-FAK ΔC14 (ΔC14). Their effects on cell cycle progression were evaluated by measuring BrdU incorporation, as described previously [6]. Consistent with our previous results [6], Fig. 2A shows that overexpression of ΔC14 inhibited cell cycle progression by approximately 70% whereas the FAK mutant ΔC14F did not significantly inhibit cell cycle progression. Interestingly, the ΔC14A mutant inhibited cell cycle progression as effectively as the ΔC14 mutant. Comparable inhibition of cell cycle progression by the ΔC14A mutant and the ΔC14 mutant was also observed using NIH 3T3 cell lines with inducible expression of the mutants (data not shown). These results indicated that the FAK/PI3K complex did not play a major role in the cell cycle regulation and that disruption of the FAK complexes with Src and potentially other signaling molecules (by the ΔC14A mutant) was sufficient to block cell cycle progression.

Our previous studies suggested that the inhibition of cell cycle progression by ΔC14 was mediated by its inhibition of adhesion-induced Erk activation due to its disruption of endogenous FAK signaling complexes [6]. To examine whether ΔC14A inhibited cell cycle progression through the same mechanism, we tested the effect of ΔC14A on endogenous Erk1/2 in the inducible NIH 3T3 cells. Lysates were prepared from cells under induced conditions that had been replated on fibronectin. They were then analyzed for adhesion-induced Erk activity using anti-phospho-Erk antibodies, as shown in Fig. 2B. Consistent with previous observations [6], overexpression of ΔC14 inhibited Erk activation compared to Mock cells. Consistent with its lack of inhibition of cell cycle progression, expression of ΔC14F did not reduce Erk activation presumably due to its lack of effects on either FAK/PI3K or FAK/Src complexes. Interestingly, expression of ΔC14A also inhibited Erk activation to a similar extent as ΔC14. Comparable expression levels of various FAK mutants were confirmed by immunoblotting of the lysates with anti-HA (lower panel). These results suggested that ΔC14A functioned to inhibit cell cycle progression in a manner similar to the ΔC14 mutant. They also suggested that disruption of the endogenous FAK complexes with Src and potentially other signaling molecules (by ΔC14A) could be sufficient to inhibit Erk activation in response to cell adhesion.

Recent studies suggested that Y397 of FAK also served as a binding site for Grb7 [17]. To assess whether the FAK/Grb7 complex contributes to regulation of cell cycle progression, we examined association of the D395A FAK mutant with Grb7.

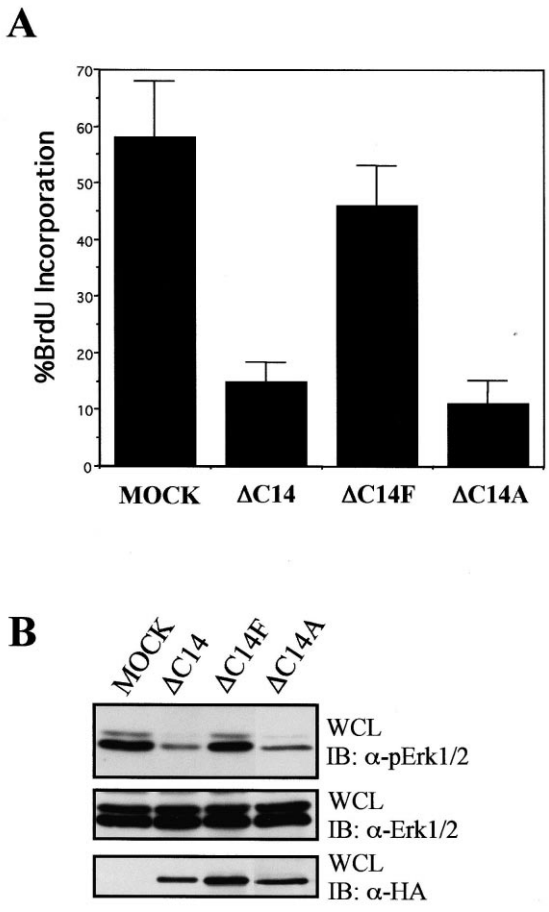

Fig. 2. PI3K association with FAK is not needed for FAK-regulation of the cell cycle or Erk1/2 activity. A: NIH 3T3 cells were transiently transfected with the indicated constructs and analyzed for DNA synthesis, as described in Section 2. Results show the mean+S.E.M. for at least three independent experiments. B: Lysates were prepared from NIH 3T3 cells overexpressing FAK and its mutants, as indicated. They were analyzed by immunoblotting with α-pErk (top panel), α-Erk (middle panel) or α-HA (bottom panel). Results are representative of two independent experiments.

293T cells were transiently co-transfected with pDHGST-Grb7 encoding Grb7 fused to GST at the N-terminus (GST–Grb7) and pCDM8-FAK (WT), pCDM8-FAK Y397F (Y397F) or pCDM8-FAK D395A (D395A). Two days after transfection, cells were lyzed and GST–Grb7 complexes were precipitated with glutathione-coupled agarose beads. After washing, the bound proteins were separated using SDS–PAGE and blotted with mAb KT3 to detect the epitope-tagged FAK or its mutants (Fig. 3A, upper panel). Consistent with the previous results [17], FAK, but not the Y397F mutant, associated with Grb7 in transfected 293 cells. Interestingly, the D395A mutant did not associate with Grb7. The specificity of the association was verified by the lack of co-precipitation of FAK when it was co-transfected with the pDHGST vector (GST) alone. Similar expression levels for FAK and mutants were confirmed by blotting whole cell lysates with anti-FAK (Fig. 3A, lower panel). These results indicated that the D395A mutation disrupted FAK association with Grb7. Therefore, the ΔC14A mutant is not expected to compete with endogenous FAK for Grb7 binding (thus will not disrupt the endogenous FAK/Grb7 complex). The inhibi-

tion of Erk activation and cell cycle progression by ΔC14A (see Fig. 2) suggested that the FAK/Grb7 complex (even together with the FAK/PI3K complex) is not sufficient for these events, and further highlights the importance of the FAK/Src complex.

We have previously established a NIH 3T3 cell line with inducible expression of the Grb7 SH2 domain alone [17]. Induction of the Grb7 SH2 domain resulted in a decrease in cell migration on FN, suggesting that it can function in a dominant negative manner to inhibit the endogenous FAK/Grb7 complex, which is involved in cell migration. In contrast, we did not observe any differences in BrdU incorporation of the cell line under induced or uninduced conditions (Fig. 3B). This provides further support for the above notion that the FAK/Grb7 complex is not involved in the regulation of cell cycle progression.

The formation of FAK/Src complex allows Src phosphorylation of FAK-bound Cas [22,23] and Y925 of FAK leading to its association with Grb2 [19,20]. To further evaluate the mechanisms of cell cycle regulation by the FAK/Src complex, we investigated the role of FAK association with Cas and Grb2 in the cell cycle regulation. Oktay et al. [10] have shown previously that inhibition of Cas/Crk complex could block FAK-dependent cell cycle progression. Although they suggested that Cas phosphorylation and its complex with Crk is required for FAK regulation of cell cycle progression, these results did not address the potential importance of direct association of FAK with Cas. The potential role of FAK/Grb2 association in cell cycle progression is unknown, although this complex has been suggested to play a role in Erk activation by FAK [19]. NIH 3T3 cells were transiently transfected with pKH3-FAK Y925F (Y925F) or pKH3-FAK P712/715A (P712/715A), which do not associate with Grb2 or Cas, respectively, and the effects of these mutants on cell cycle progression were evaluated by measuring BrdU incorporation, as described previously [6]. Fig. 4 shows that overexpression of either P712/715A or Y925F mutants inhibited cell cycle progression by approximately 50% when compared with the Mock transfected cells. Transfection of pKH3-FAK COMBO (COMBO; P712 and P715 mutated to A and Y925 mutated to F) resulted in a more significant inhibition than either of the single mutants. Similar results were obtained in stable NIH 3T3 cell lines with inducible expression of the FAK P712/715A, Y925F or the combo mutant (data not shown). Together, these results suggested that FAK association with both Cas and Grb2 are involved in the regulation of cell cycle progression and the two complexes might work in parallel pathways.

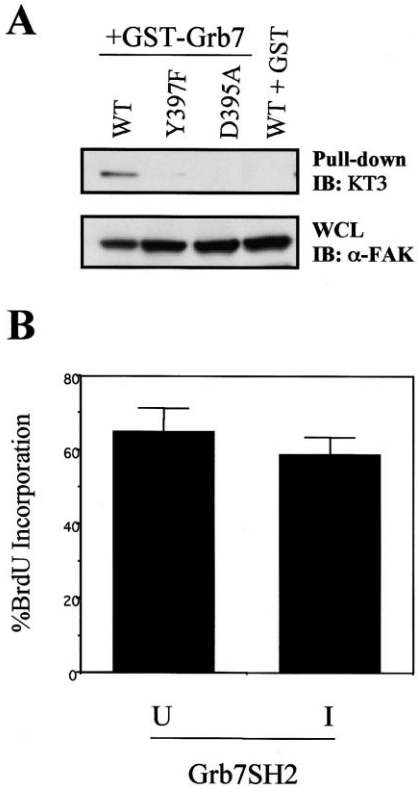

Fig. 3. FAK association with Grb7 is not required for cell cycle progression. A: HEK 293T cells were co-transfected with GST–Grb7 or GST and FAK or its mutants as indicated. Lysates were then prepared from these cells, incubated with glutathione agarose (Pull-down) and analyzed by immunoblotting with KT3 (top panel). Aliquots of lysates were also analyzed by immunoblotting with α-FAK directly (lower panel). B: The Grb7 SH2 inducible cell line was cultured in the presence (U) or absence (I) of tetracycline to repress or induce the expression of Grb7 SH2. These were analyzed for DNA synthesis, as described in Section 2. Results show the mean+S.E.M. for at least two independent experiments.

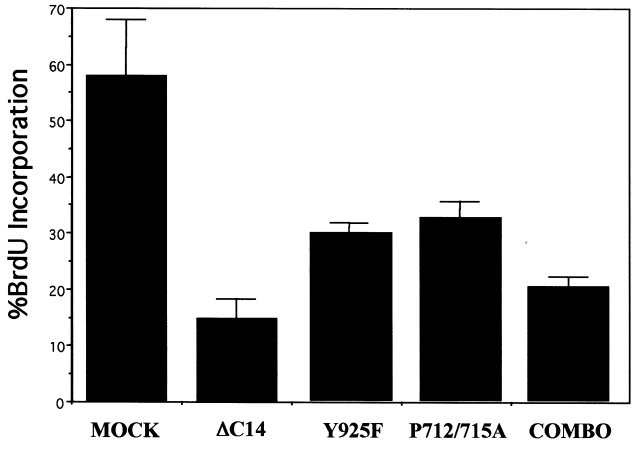

Fig. 4. Grb2 and Cas association with FAK are required for cellular proliferation. NIH 3T3 cells were transiently transfected with the indicated constructs and analyzed for DNA synthesis, as described in Section 2. Results show the mean+S.E.M. for at least three independent experiments.

4. Discussion

Integrin signaling through FAK has been shown to regulate a variety of cellular processes including cell spreading and migration, cell survival and apoptosis, and cell cycle progression [1,2]. It has been suggested that FAK regulated these cellular functions through its interaction with a variety of other signaling molecules. However, the specific contributions of distinct FAK signaling complexes toward FAK-regulation of these cellular processes are poorly understood. Using specific FAK mutants deficient in binding to specific partners, we showed that FAK association with PI3K or Grb7 is not involved in the regulation of cell cycle progression by FAK, while the FAK/Src complex plays an important role in this process. Furthermore, we found that FAK interactions with

Cas or Grb2 are both required for the regulation of cellular proliferation.

Previous studies from our laboratory have shown that a mislocalized FAK mutant, ΔC14, inhibited cell cycle progression. This inhibition was dependent on Y397 of FAK [6]. Since Y397 is a binding site for a number of signaling molecules including Src [13,14], PI3K [15], Grb7 [17] and PLCγ1 [16], these studies implicated a role for FAK complexes with these molecules although the contribution by each signaling complex (e.g. FAK/Src versus FAK/PI3K) was not determined. We have recently generated a mutation (D395A) in FAK that selectively disrupts the association of FAK with PI3K, while leaving all Src-dependent elements of FAK function unchanged [9]. Additional analysis here indicates that the D395A mutation also disrupts FAK/Grb7 association (Fig. 3). Use of this mutation in the context of the ΔC14 background (i.e. ΔC14A) allowed us to assess the roles of the FAK/Src complex versus FAK association with PI3K and Grb7. We found that ΔC14A was able to inhibit cell cycle progression as effectively as the ΔC14 mutant (Fig. 2), even though it could not compete with the endogenous FAK for binding PI3K and Grb7. If either the FAK/PI3K or the FAK/Grb7 complexes were playing a role in cell cycle progression, free PI3K and/or Grb7 would be expected to relieve, at least partially, the inhibition of cell cycle progression by ΔC14. In addition, a dominant negative Grb7 mutant [17] failed to affect DNA synthesis (Fig. 3B). Together these results suggested that FAK/PI3K and FAK/Grb7 were not involved in FAK-regulated cell cycle progression. They provided the first example that particular FAK signaling complexes (e.g. FAK/PI3K) are involved in a subset ([17], this study), but not all, of FAK-regulated cellular functions.

Results presented here suggested that the FAK/Src complex might regulate cell cycle progression through multiple downstream pathways. We found that FAK interaction with Cas and Grb2, both downstream events of FAK/Src complex formation [19,20,22,23], were necessary for the regulation of cell cycle progression by FAK. Expression of either FAK mutant lacking binding to Cas or Grb2 each inhibited cell cycle progression (Fig. 4). A previous report has suggested that FAK signaled through Cas to JNK in the regulation of cell cycle progression [10]. However, these experiments did not investigate FAK/Cas association directly. Reagents that block total JNK or Cas function may be interfering with cell cycling pathways that do not involve FAK. Furthermore, Cas-bound Crk can associate with the GEF C3G [22] that can potentially activate the Ras/Erk cascade, thus a role for Cas in signaling to Erk cannot be discounted. Nonetheless, FAK/Src signaling through Cas could potentially regulate cell cycling independently of Erk, signaling through JNK. Indeed, preliminary data from our laboratory suggests that FAK/Cas association is required for adhesion-stimulated JNK2 activation (data not shown).

The mechanism by which the FAK Y925F mutant lacking Grb2 binding inhibited cell cycle progression is not clear at present. Previous studies suggested that FAK association with Grb2 at Y925 contributes to Erk activation in cell adhesion [29] or upon HGF-stimulation [30]. Thus, it is possible that Grb2 binding to Y925 of FAK plays a role in regulating cell cycle progression through its effects on Erk activation. Alternatively, Grb2 binding to FAK at Y925 may couple FAK to other cellular proteins such as dynamin [31], which may contribute to cell cycle regulation in an Erk-independent manner. Dynamin is involved in the regulation of endocytosis and receptor internalization [32], and Grb2 association with dynamin has been shown to mediate signaling in response to growth factors distinct from that of the Grb2/SOS complex [33]. Finally, we cannot exclude the possibility that FAK may interact with other proteins through Y925, which may contribute to FAK/Src-dependent regulation of cell cycle progression. Further studies will be necessary to clarify the various possibilities.

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