Association of Focal Adhesion Kinase with Tuberous Sclerosis Complex 2 in the Regulation of S6 Kinase Activation and Cell Growth*

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Tuberous sclerosis complex 1 (TSC1) and TSC2 tumor suppressor proteins have been shown to negatively regulate cell growth through inhibition of the mammalian target of rapamycin (mTOR) pathway. Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that plays a critical role in integrin signaling. Here we identify a novel interaction between FAK and TSC2 and show that TSC2 is phosphorylated by FAK. Furthermore, we show that overexpression of FAK kinase dead mutant inhibits the phosphorylation of ribosomal S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein-1, two key mTOR (mammalian target of rapamycin) downstream targets, and negatively regulates the cell size and that FAK regulation of S6K phosphorylation is through TSC2. Finally, we provide data that FAK plays a positive role in cell adhesion-induced S6K phosphorylation, whereas TSC2 is required for cell suspension-induced S6K inactivation. Together, these results suggest that FAK might regulate S6K activation and cell size through its interaction with and phosphorylation of TSC2 and also provide a previously unappreciated role of TSC2 in the regulation of mTOR signaling by cell adhesion.

Cell growth, the increase of cell size or cell mass, is a fundamental biological process and is mainly regulated by protein synthesis. Considerable bodies of evidence indicate that the tuberous sclerosis complex (TSC)2-mammalian target of rapamycin (mTOR) signaling cascade plays an essential role in the regulation of cell growth (1–6). TSC1 and TSC2 are both tumor suppressor genes responsible for tuberous sclerosis, which is characterized by the formation of hamartomas in a wide range of tissues (4). TSC1 and TSC2 contain coiled-coil regions and can form a physical and functional complex in vivo (7, 8). Although TSC1 has no known enzymatic activity, TSC2 contains a carboxyl-terminal GAP domain for the small G protein Rheb. Recent studies have shown that TSC1-TSC2 complex functions as a potent negative regulator of cell growth mainly by their inhibition of mTOR and its targets ribosomal S6 kinase (S6K) and eukaryotic initiation factor 4E binding protein-1 (4EBP-1), which play essential roles in the regulation of protein synthesis and cell size (9–15).

Phosphorylation plays critical roles in the regulation of signal transduction. TSC2 initially was shown to undergo both tyrosine and serine phosphorylation in response to various stimuli (16). Indeed, recent studies identified multiple serine/threonine kinases capable of phosphorylating TSC2 and regulating its function in mTOR signaling, including Akt, AMP-activated protein kinase, p90 ribosomal S6 kinase 1, and extracellular signal-regulated kinase (13, 17–20). However, the tyrosine kinase responsible for TSC2 tyrosine phosphorylation has not been reported so far.

Cell adhesion signaling has been shown to play key roles in a number of important cellular processes, including cell migration, cell survival, cell proliferation, and cell growth (21, 22). In contrast to our extensive understanding of the mechanisms that regulate cell migration, cell survival, and cell proliferation through cell adhesion signaling, relatively little is known about the mechanisms by which cell adhesion regulates cell growth. Focal adhesion kinase (FAK) is a 125-kDa protein-tyrosine kinase that plays a major role in cell adhesion-mediated signal transduction. Numerous studies have shown that FAK functions to regulate cytoskeleton organization, cell migration, cell proliferation, and cell survival through its interaction with and phosphorylation of other downstream signaling molecules (23, 24). FAK has also been shown to play a role in cell adhesion-stimulated S6K activation, although the underlying mechanism remains unknown (25). In an effort to further explore potential mechanisms of FAK regulation of S6K activation and cell size, we searched for other FAK-interacting proteins involved in the regulation of cell size.

In this study we show a novel interaction between FAK and TSC2. We show that TSC2 could be phosphorylated by FAK in vivo. Furthermore, we demonstrate that overexpression of FAK kinase dead mutant inhibits the phosphorylation of S6K and 4EBP-1 and negatively regulates the cell size and that FAK regulation of S6K phosphorylation is through TSC2. Finally, we provide evidence that FAK plays a role in cell adhesion-induced signaling.
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S6K phosphorylation, whereas TSC2 is required for cell suspension-induced S6K inactivation.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—Affinity-purified antibody against GST was prepared from anti-GST serum using GST immobilized on glutathione-Sepharose as an affinity matrix. The mouse monoclonal FAK antibody, the mouse monoclonal phosphotyrosine antibody (4G10), and the rabbit polyclonal FAK site-specific antibody against phosphorylated Tyr-397 (PY397) were obtained from Upstate Biotechnology (Lake Placid, NY). The rabbit polyclonal α-HA (Y11) antibody, the mouse monoclonal α-c-Myc tag (9E10) antibody, rabbit polyclonal TSC2 antibody, rabbit polyclonal α-FAK (C20) antibody, and rabbit polyclonal S6K antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit polyclonal Thr-389 phospho-S6K, rabbit polyclonal S6, rabbit polyclonal Ser-240/244 phospho-S6, rabbit polyclonal 4EBP-1, and rabbit polyclonal Thr-37/46 phospho-4E-BP1 antibodies were from Cell Signaling Inc. (Beverly, MA). Anti-HA-conjugated agarose beads, glutathione-agarose beads, protein A-Sepharose, human plasma fibronectin (EN), and mouse monoclonal FLAG antibody were purchased from Sigma.

Plasmid DNA Construction—HA-tagged expression vectors pKH3, pKH3-FAK, pKH3-FAK-Y397F, pKH3-FAK-kd (kinase dead), pKH3-NT-FAK, pKH3-KD-FAK (kinase domain), pKH3-CT-FAK, pKH3-KC-FAK, Myc-tagged expression vector pPHAN, pPHAN-FAK, GST-tagged expression vector pDHGST, and pDHGST-FAK have been described previously (35, 36). Expression vectors encoding HA-TSC2, HA-TSC2 1–1320, HA-TSC2 1–1080, HA-TSC2 1–608, HA-TSC2 1080–1765, GST-TSC2, Myc-TSC1, HA-S6K, and FLAG-4EBP-1 have been described previously (13, 37). TSC2 609–1080-amino acid segment was amplified by primers 5′-CCCCATCGATTTAGGCCCCACGGGACACCTGCTGCCC-3′ (forward) and 5′-CGCGATCCACGATCCCGTGCAGGCCTTTGAC-3′ (reverse), digested with BamHI and EcoRI, and ligated with pKH3 digested with the same enzymes to generate pKH3-TSC2 609–1080. pKH3-TSC2 609–1080 was then digested by CiaI and filled in by Klenow enzyme followed by BamHI digestion to release TSC2 609–1080 segment, which was ligated into pGEX2T digested by BamHI and Smal to generate pGEX2T-TSC2 609–1080.

The RNAi vector pBS-U6 is a gift from Y. Shi (Harvard Medical School, Boston, MA). Construction of TSC2 RNAi vector was achieved in two separate steps as previously described (38). Briefly, a 22-nucleotide oligo (oligo 1) was first inserted into the pBS-U6 vector digested with Apal (blunted) and HindIII. The inverted motif that contained the 6-nucleotide spacer and 5 T residues (oligo 2) was then subcloned into the HindIII and EcoRI sites of the intermediate plasmid to generate pBS-U6-TSC2.

The sequence of oligo1 is 5′-GGCACATTGTTGAAGTTGCACAAA-3′ (forward) and 5′-AGCTTTTGGCAGATCCACAATTGCC-3′ (reverse); the sequence of oligo2 is 5′-AGCTTTTGCGACTTCACAAATCTGCCCCTTTTG-3′ (forward) and 5′-AATTCCAAAAAGGGCAAGATTTGTAAGTCGGAAA-3′ (reverse). The RNAi targeting sequences were all analyzed by BLAST search to ensure that they did not have significant sequence homology with other genes.

Cell Culture, Transfection, and Adenovirus Infection—The TSC2−/− LEF cells that stably express either TSC2 (TSC2−/− + TSC2 cells) or an empty vector (TSC2−/− + Vec cells) were generous gifts from Dr. Kun-Liang Guan of University of Michigan and were maintained in DMEM supplemented with 10% FBS containing 0.5 mg/ml G418, as previously described (18). SYF cells were generous gifts from Drs. L. Cary, R. Klinghoffer, and P. Soriano of Fred Hutchinson Cancer Research Center and were maintained in DMEM with 10% FBS. The FAK KO MEFs were a generous gift of Dr. D. Ilic (University of California, San Francisco) and were maintained in DMEM supplemented with 10% FBS. 293T cells and HeLa cells were cultured in DMEM supplemented with 10% FBS. NIH3T3 cells were cultured in DMEM supplemented with 10% calf serum.

Cells were transfected with mammalian expression plasmids as indicated using Lipofectamine™ following the manufacturer’s instructions. Experiments were conducted 24–48 h after transfection.

The recombinant adenoviruses encoding FAK kinase dead mutant (Ad-FAK kd), RNAi adenoviruses Ad-FAK RNAi, and Ad-Control RNAi were used as previously described (39, 40). Cells were infected by Ad-FAK kd or Ad-GFP for 36 h before the experiments and infected by Ad-FAK RNAi or Ad-Control RNAi for 72 h before the experiments.

For FN replating experiment, the growing cells were briefly trypsinized, resuspended in Dulbecco’s modified Eagle medium containing a final concentration of 0.5 mg/ml soybean trypsin inhibitor, washed twice in Dulbecco’s modified Eagle’s medium, and held in suspension for 60 min. Indicated cells were lysed. Cells replated on FN were added to plates coated with 10 µg/ml FN for different periods of time as indicated.

Immunoprecipitation and Western Blotting—For most experiments subconfluent cells were washed twice with ice-cold phosphate-buffered saline (PBS) and then lysed with 1% Nonidet P-40 lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 1% Nonidet P40, 10% glycerol, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, and 2 µg/ml leupeptin). For experiments to detect in vivo phosphorylation of TSC2, 0.5 mM pervanadate was used in lysis buffer instead of sodium vanadate as phosphatase inhibitor. Briefly, 500 mM sodium vandate was mixed with 500 mM H2O2 (1:1) and incubated at room temperature for 10–15 min before adding to lysis buffer (1:250 dilution). Lysates were cleared by centrifugation for 10 min at 4 °C, and total protein concentration was determined using Bio-Rad protein assay. Immunoprecipitations were carried out by incubating cell lysates with HA-conjugated agarose beads or glutathione-agarose beads for more than 3 h at 4 °C. To detect endogenous protein-protein interaction, immunoprecipitations were carried out by incubating cell lysates with appropriate antibodies as indicated for more than 3 h at 4 °C followed by incubation for another 3 h with protein A-Sepharose. After washing 3–5 times with lysis buffer, immune complexes were resolved using SDS-PAGE. Western blotting was carried out using horseradish peroxidase-conjugated IgG as a secondary antibody and the Amersham Biosciences ECL system for detection.
Preparation of GST Fusion Proteins and in Vitro Binding Assays—GST-TSC2 609–1080 fusion proteins were purified and immobilized on glutathione-agarose beads and then incubated with recombinant His-tagged FAK in 300 μl of 1% Nonidet P-40 lysis buffer for more than 3 h at 4 °C. The samples were then washed five times with lysis buffer, boiled in SDS buffer, resolved by SDS-PAGE, and analyzed by Western blotting with anti-GST and anti-FAK antibody.

Cell Size Assay—To determine cell size and DNA content, FACS analysis with Cell Quest software was performed as previously described (18, 38). Briefly, 293T cells grown in 60-mm plates were transfected with various expression vectors. The next day the confluent cells were split in 10-cm dishes (1:4 split). The cultured medium was changed every 24 h until confluent. The cells usually became confluent for harvest after 2–3 days. To harvest cells, plates were washed once with PBS and incubated at 37 °C for 2 min in 1 ml of trypsin-EDTA. Cells were gently pipetted off the plate with PBS, 10% FBS, transferred to 15-ml conical tubes, centrifuged for 3 min at 1000 rpm, and washed once with PBS, 1% FBS, and the final cells pellets were resuspended with 1 ml of PBS, 1% FBS. Resuspended cells were fixed on ice by adding 3 ml of cold 100% ethanol (75% final) while vortexing. Fixed cells were stored at 4 °C until the time of analysis. Immediately before analysis on FACS, the fixed cells were centrifuged at 1500 rpm for 3 min, washed once with PBS, 1% FBS, resuspended in 1 ml of PBS containing 0.1% Triton X-100 and 100 μg/ml RNase A, and incubated at 37 °C for 20–30 min. The cells were stained by propidium iodide for FACS analysis to determine the cells size in G1-phase and G2/M-phase populations.

For MEF cells size analysis, completely trypsinized cells were seeded in a 10-cm dish at 20% confluency and then cultured with DMEM, 10% FBS to 90% confluency. The cells were analyzed by FACS as described above.

RESULTS

Association of FAK with TSC2—To investigate a potential interaction between FAK and TSC2, we first co-transfected the vector encoding Myc-tagged FAK with HA-tagged TSC2 or HA empty vector into 293T cells. Cell lysates were then immunoprecipitated with anti-HA antibody and followed by Western blotting with anti-Myc antibody. Fig. 1A shows that Myc-FAK was co-immunoprecipitated with HA-TSC2 but not detected in the immunoprecipitates of control lysates from cells co-transfected with HA empty vector. We could also detect TSC2 in FAK immunoprecipitates by reciprocal IP. As shown in Fig. 1B, the vector encoding GST-tagged TSC2 and HA-tagged FAK or HA empty vector were co-transfected into 293T cells followed by immunoprecipitation by anti-HA antibody. GST-TSC2 was found to co-immunoprecipitate with HA-FAK but not detected in the immunoprecipitates of control lysates from cells co-transfected with HA empty vector. Consistent with these transfection studies, we could also detect the interaction of endogenous FAK and TSC2 in NIH3T3 cells. As shown in Fig. 1C, FAK was detected in anti-TSC2 immunoprecipitates from attached cells but not suspended cells. Together, these results suggest that TSC2 could interact with FAK in vivo.

It has been shown that TSC1 and TSC2 form a stable complex in vivo (7, 8). Thus, we tested whether FAK could also interact with TSC1. We co-transfected vector encoding GST-tagged FAK or GST empty vector into 293T cells with vectors encoding Myc-TSC1 and HA-TSC2, either individually or in combination. Fig. 1D shows that FAK could co-immunoprecipitate with TSC2 regardless of TSC1 co-expression (lanes 1 and 3). TSC1 could also be detected in FAK immunoprecipitates when TSC2 was co-expressed (lane 1). However, FAK did not apparently interact with TSC1 with-
out TSC2 co-expression (lane 2). These data suggest that FAK could form a ternary complex with both TSC1 and TSC2 and that FAK indirectly interacts with TSC1 through its association with TSC2.

**Characterization of the Binding Regions in FAK and TSC2**—To determine the TSC2 binding site on FAK, we co-expressed a GST-tagged TSC2 vector with the vector encoding HA-tagged wild-type FAK or various FAK mutants (Fig. 2A) in 293T cells. Immunoprecipitations were performed with an anti-HA antibody followed by Western blotting with an anti-GST antibody. Fig. 2C shows that, as expected, TSC2 was co-immunoprecipitated with HA-FAK. TSC2 was also co-immunoprecipitated with FAK Y397F and kd (kinase dead) mutants, suggesting that neither tyrosine 397 phosphorylation nor kinase activity of FAK is required for FAK association with TSC2. Further mapping experiments by using several FAK truncation mutants demonstrated that FAK KD (kinase domain) and KC domain associated with TSC2, whereas FAK NT domain and CT domain showed no binding to TSC2 (Fig. 2, A and C). These results suggest that the kinase domain of FAK mediates its interaction with TSC2.

A similar strategy was used to define the FAK binding region within TSC2 by co-expression of Myc-tagged FAK vector with the vector encoding various HA-tagged TSC2 truncation mutants (Fig. 2B) in 293T cells followed by immunoprecipitations with anti-HA antibodies. As showed in Fig. 2D, TSC2 1081–1765 fragment, which contains Rheb-GAP domain, did not apparently interact with FAK, whereas TSC2 1–1080 fragment could interact with FAK as well as full-length and the 1–1320 fragment of TSC2, suggesting that Rheb-GAP domain in TSC2 is neither sufficient nor required for TSC2 interaction with FAK. Furthermore, TSC2 1–608 fragment showed no obvious interaction with FAK (Fig. 2, B and D). Thus, these results suggest that 609–1080 amino acids of TSC2 are required for TSC2 interaction with FAK. Last, we found that His-tagged recombinant FAK protein was sufficient to bind to recombinant GST fusion protein containing TSC2 609–1080 amino acids, but not GST alone, in vitro (Fig. 2E), suggesting that 609–1080 amino acids of TSC2 mediates a direct interaction between FAK and TSC2.

**FAK Phosphorylation of TSC2**—The identification of the interaction between FAK and TSC2 raises the possibility of tyrosine phosphorylation of TSC2 by FAK. To determine whether TSC2 serves as a phosphorylation substrate for FAK, we co-transfected HA-tagged TSC2 vector with Myc-tagged FAK, Myc-tagged FAK kinase dead mutant (FAK kd), or Myc empty vector control into 293T cells. Cell lysates were then immunoprecipitated with anti-HA antibody followed by Western blotting with anti-phosphotyrosine antibody 4G10. We observed that TSC2 could be phosphorylated by WT FAK, but not by FAK kd mutant, suggesting that TSC2 serves as a phosphorylation substrate for FAK in vivo (Fig. 3A). Interestingly, overexpression of FAK kd mutant even decreased the basal phosphorylation level of TSC2 (Fig. 3A). Because FAK kd interacted with TSC2 as efficiently as FAK WT (see Fig. 2B), FAK kd mutant might function as a dominant negative mutant to compete with endogenous FAK for interaction with TSC2 and, thus,
decreased the basal phosphorylation level of TSC2. Finally, phosphorylation of TSC2 by FAK was also observed in SYF cells that are deficient in the expression of Src family kinases Src, Yes, and Fyn (26) (Fig. 3B), suggesting that phosphorylation of TSC2 by FAK is independent of the Src family kinases.

FAK Regulation of S6K Phosphorylation and Cell Size through TSC2—Recent studies have shown that TSC1/TSC2 complex negatively regulates cell size through inhibition of mTOR-S6K pathway (9–15). Interestingly, it has been shown that overexpression of FAK-related non-kinase inhibited cell adhesion-mediated activation of S6K, suggesting that FAK might play a role in cell adhesion-mediated activation of S6K (25). Thus, it is possible that FAK might regulate S6K phosphorylation through TSC2. To examine this possibility, 293T cells were co-transfected with HA-FAK or HA-FAK kd mutant, or empty vector along with the vector encoding HA-tagged S6K. Cell lysates were then prepared and analyzed by Western blotting with various antibodies as indicated. Aliquots of the lysates (WCL) were also analyzed directly.

Fig. 4 shows that, although overexpression of FAK kd decreased S6K phosphorylation (compare lanes 1 and 2), the negative effect on S6K phosphorylation by FAK kd was abolished under TSC2 knock-down condition (compare lanes 3 and 4). To further test the functional relationship between FAK and TSC2, we overexpressed FAK kd via adenoviral infection in TSC2−/− LEF cells that stably express either TSC2 (TSC2−/− + TSC2 cells) or an empty vector (TSC2−/− + VEC cells). Fig. 5B shows that overexpression of FAK kd caused a reduction in endogenous S6K phosphorylation in TSC2 reexpressing cells but not in TSC2−/− cells. Finally, Fig. 5C shows that overexpression of FAK KD (which is capable of interacting with TSC2), but not NT domain (which does not interact with TSC2), functioned as a dominant negative mutant to suppress S6K phosphorylation. Collectively, these results suggest that FAK functions upstream of TSC2 to regulate S6K phosphorylation and that FAK up-regulates mTOR signaling and cell size potentially through its interaction with and phosphorylation of TSC2.

The Roles of FAK and TSC2 in the Regulation of S6K Phosphorylation by Cell Adhesion—We further examined whether FAK plays any physiological role in the regulation of S6K phosphorylation by cell adhesion signaling, as a number of studies have showed that FAK can be potently activated by cell adhesion, which can also promote S6K phosphorylation (23–25). We, thus, examined the effect of S6K phosphorylation by cell adhesion upon endogenous FAK knock-down by FAK RNAi. HeLa cells were infected with adenoviruses Ad-FAK RNAi or Ad-Control RNAi. Cell lysates were prepared from suspended cells or cells replated on fibronectins for different periods of time and followed by Western blotting with phosho-S6K. Fig. 6A shows that cell adhesion-induced S6K phosphorylation was attenuated in FAK RNAi-treated cells compared with control RNAi-treated cells. We further examined whether overexpression of FAK WT or kinase dead mutant would have any effect on S6K phosphorylation under cell suspension and adhesion conditions. Fig. 6B shows that overexpression of FAK kinase dead mutant suppressed the cell adhesion-induced S6K phosphorylation. Interestingly, overexpression of FAK wild type increased S6K phosphorylation under suspension conditions, although it did not affect S6K phosphorylation during cell
adhesion. Taken together, our results suggest that FAK plays a positive role in cell adhesion-induced S6K phosphorylation.

TSC2 has been shown to function as the integration point to receive signaling from several stimuli, such as growth factors, nutrients, intracellular energy levels, and hypoxia, to regulate S6K phosphorylation (11, 18, 27, 28). However, its role in the regulation of S6K function by cell adhesion has not been addressed. Our observation that FAK plays a positive role in cell adhesion-induced S6K phosphorylation suggests that TSC2 might play a role in S6K inactivation by cell suspension. We, thus, compared S6K phosphorylation in TSC2<sup>+/+</sup> + TSC2 cells or TSC2<sup>-/-</sup> + VEC cells upon cell suspension. Fig. 6C shows that cell suspension treatment caused a dramatic decrease in S6K phosphorylation in TSC2 reexpressing (TSC2<sup>-/-</sup> + TSC2) cells; however, this effect was largely abolished in TSC2<sup>-/-</sup> cells. Similarly, we found that cell suspension down-regulated S6 and 4EBP-1 phosphorylation in TSC2 reexpressing cells but failed to decrease S6 and 4EBP-1 phosphorylation in TSC2<sup>-/-</sup> cells (Fig. 6C). Together, these results strongly suggest that TSC2 plays an important role in cell suspension-induced S6K inactivation.

**DISCUSSION**

FAK is an important mediator of signal transduction pathways initiated by integrins in cell migration, survival, and cell cycle regulation (23, 24). Here we provide several lines of evidence to suggest that FAK also plays an important role in cell size control. First, overexpression of FAK kd mutant inhibits the phosphorylation of S6K and 4EBP-1 and decreases cell size in 293T cells. Second, FAK regulation of S6K activation is abolished in TSC2 knockdown or knock-out background. Our study, thus, strongly suggests that FAK functions upstream of TSC2 and mTOR to regulate S6K activation and cell size. In addition, we show that FAK plays a positive role in cell adhesion-induced S6K phosphorylation, whereas TSC2 is required for cell suspension-induced S6K inactivation. Finally, we identify a novel cell adhesion-dependent interaction between FAK and TSC2 and show that TSC2 could be phosphorylated by FAK. Importantly, we show that the association of FAK KD and NT domains with TSC2 correlated with their ability to regulate S6K phosphorylation, suggesting that FAK regulates cell size possibly through its interaction with and phosphorylation of TSC2. Taken together, our data together suggest the following model. In suspended cells, because FAK is inactivated, FAK-mediated inhibition of TSC2 is relieved, and TSC2 functions to suppress S6K phosphorylation and cell growth. Cell adhesion activates FAK and promotes FAK-TSC2 interaction; activated FAK then inactivates TSC2 through its interaction with TSC2, which lead to increased S6K phospho-
ralyation and cell growth. This model also explains our findings that TSC2 is required for kd FAK inhibition of S6K phosphorylation in adhered cells and that TSC2 is required for the decrease in S6K phosphorylation upon cell suspension.

It has been shown that integrin-mediated cell adhesion stimulated S6K phosphorylation and that overexpression of FAK-related non-kinase caused a significant reduction in the integrin-mediated activation of S6K, suggesting that FAK might play a role in cell adhesion-mediated S6K activation (25). However, the mechanism by which FAK regulates S6K activation was not addressed in the study. Furthermore, because S6K activation has also been shown to play an important role in other cellular processes, such as cell proliferation (1), it is not clear whether FAK-mediated S6K activation plays any role in cell size control. Our study presented here is consistent with the previous observation and also provides several novel mechanistic insights about FAK function in the regulation of cell size.

A number of recent studies have shown that TSC1-TSC2 complex functions to negatively regulate cell size by their inhibition of mTOR and its targets S6K and 4E-BP1 (9–15) and that a variety of extracellular and intracellular signals such as growth factors, energy level, nutrient level, and hypoxia regulate S6K phosphorylation through TSC1-TSC2 complex (11, 18, 27, 28). Our study here showed for the first time that TSC2 also plays a critical role in the regulation of S6K phosphorylation by cell adhesion signaling. Integrin-mediated cell adhesion signaling has been shown to play a critical role in a number of biological processes, including angiogenesis (21, 22). Although most studies have focused on the roles of integrin-regulated cell survival, proliferation, and migration pathways in angiogenesis, recent studies have begun to reveal the potential role of protein syn-
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thesis and cell growth in the regulation of angiogenesis by integrin signaling. For example, it has been shown that tumstatin, a potent angiogenesis inhibitor, specifically inhibits mTOR signaling and cap-dependent protein synthesis in vascular endothelial cells through tumstatin interaction with integrin αV/β3, which might mediate the anti-angiogenic function of tumstatin (29). It is possible that the dysregulation of cell adhesion-mediated angiogenesis might play a role in pathobiology of tuberous sclerosis. Intriguingly, TSC patients develop cutaneous angiofibromas and renal angiomyolipomas, and TSC1, TSC2 heterozygous mice develop liver hemangiomas, all of which are highly vascular (4, 30–33).

The molecular mechanisms by which the function of TSC is modified by various stimuli to regulate cell size have been extensively studied recently. Most studies indicate that phosphorylation of TSC2 by other kinases plays a major role in the regulation of TSC function. For example, it has been shown that TSC1–TSC2 complex could be regulated by growth factor signaling through TSC2 phosphorylation by Akt, extracellular signal-regulated kinase, and p90 ribosomal S6 kinase 1, whereas AMP-activated protein kinase-mediated TSC2 phosphorylation plays a major role in TSC regulation by energy level deprivation (13, 17–20). Furthermore, anisomyosin-induced TSC2 phosphorylation is mediated by TSC2 phosphorylation of MK2, although the functional significance of this phosphorylation is not clear (34). Although the initial study indicated that TSC2 could undergo both tyrosine and serine phosphorylation (16), all the TSC2 kinases identified so far are serine/threonine kinases, and the tyrosine and serine phosphorylation (16), all the TSC2 kinases phosphorylated by FAK through its interaction with FAK. 

References

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