Integrin-Linked Kinase Complexes with Caveolin-1 in Human Neuroblastoma Cells†

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ABSTRACT: Integrin-linked kinase (ILK) and caveolin-1 (cav-1) are implicated in the pathogenesis of cancer. Overexpression of ILK leads to altered expression of cell cycle regulators, a decreased level of cell adhesion to the extracellular matrix, a decreased level of apoptosis, *in vitro* phosphorylation of Akt, and tumor formation in nude mice. Conversely, cav-1 expression is frequently downregulated in many forms of cancer. We examined whether ILK and cav-1 interact in SHEP human neuroblastoma cells because ILK is present in caveolae-enriched membranes and contains a putative cav-binding domain. SHEP cells were stably transfected with vector, wild-type ILK (ILK-wt), kinase-deficient ILK (ILK-kd), or mutant cav-binding domain ILK (ILK-mutCavbd). Control SHEP cells and ILK transfectants express high levels of ILK and cav-1. Immunoprecipitation with anti-cav-1 co-immunoprecipitates a 59 kDa protein that is immunoreactive with the anti-ILK antibody, and this interaction is partially prevented in cells expressing ILK-mutCavbd. Cav-1 and ILK partially colocalize in SHEP cells, also supporting these data. Last, affinity chromatography with a biotinylated cav-scaffolding domain peptide precipitates ILK-wt but not ILK-mutCavbd. These data suggest that the cav-binding domain of ILK and the cav-scaffolding domain of cav-1 mediate complex formation in human neuroblastoma cells.

Neuroblastoma (NBL),[1] a cancer that accounts for approximately 8−10% of all childhood tumors, is the most common malignant disease of infancy and develops through aberrant regulation of neural crest cell differentiation (*1*). Many NBL tumors and cell lines have changes in integrin signaling (*2*), which normally modulates cell adhesion, cell spreading, and proper neuronal differentiation during development (*3*). Integrin-linked kinase (ILK), a putative oncogene (*4*), is a 59 kDa protein expressed in 33% of NBL tumors (*5*) initially isolated as a binding partner for β1-integrin (*6*). In addition to β1-integrin, ILK binds to other signaling and adapter proteins, and these interactions are important in cell adhesion, cell spreading, growth factor signaling, and cytoskeletal interactions (*7*). Within the C-terminal kinase domain, ILK has a putative caveolin-1 (cav-1) binding domain [**WSF**AVLL**W** (critical aromatic residues in bold type) (our observations)]. Cav-1 is an alleged tumor suppressor protein, since loss of expression frequently leads to transformation (*8*). The presence of a putative cav-binding domain within ILK suggests that cav-1 may modulate ILK activity and affect cellular transformation.

In this study, we show that ILK and cav-1 form a complex in SHEP human neuroblastoma cells and that mutation of the cav-binding domain of ILK (ILK-mutCavbd) partially prevents complex formation. In ILK-wt-transfected but not ILK-mutCavbd-transfected SHEP cells, anti-cav-1 antibody co-immunoprecipitates ILK. Cav-1 and ILK colocalize in SHEP cells, a fact which is altered in ILK-mutCavbd cells. Further, affinity chromatography with a biotinylated peptide generated to the cav-scaffolding domain (CSD) of cav-1 precipitates ILK-wt but not ILK-mutCavbd. These results indicate that the cav-binding domain of ILK and the CSD of cav-1 mediate complex formation.

EXPERIMENTAL PROCEDURES

Materials. Falcon brand tissue culture supplies were purchased from BD Biosciences (Bedford, MA), and chemicals were from Sigma-Aldrich Corp (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Dulbecco's Modified Eagle's Medium (DMEM) was from Invitrogen Corp. (Carlsbad, CA), and calf serum (CS) was from Hyclone (Logan, UT).

Cell Culture. SHEP human NBL cells were maintained in DMEM supplemented with 10% CS at 37 °C in a humidified atmosphere with 10% CO_2. Transfected SHEP cells were maintained in DMEM with 10% CS and 250 μg/mL G418 (Invitrogen). Cells were routinely dissociated with trypsin-EDTA (Invitrogen) for subculture.

Stable Transfection. SHEP cells were stably transfected with pcDNA3.1 or ILK-wt, ILK-kd, or ILK-mutCavbd using

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[1] Abbreviations: BCSD, N-terminal biotinylated caveolin scaffolding domain; BpTH, N-terminal biotinylated human parathyroid hormone; CEM, caveolin-enriched membrane; Cav-1, caveolin-1; CSD, caveolin-scaffolding domain; DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ILK, integrin-linked kinase; ILK-kd, kinase-deficient ILK; ILK-mutCavbd, mutant caveolin-binding domain ILK; ILK-wt, wild-type ILK; NBL, neuroblastoma.

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Lipofectamine 2000 following the manufacturer's instructions (Invitrogen). S. Dedhar (University of Toronto, Toronto, ON) kindly provided the ILK-wt and ILK-kd constructs. The vector is tagged with a V5 epitope at the C-terminal end to allow for verification of transfection. Transfected cells were selected in 500 μg/mL G418.

Caveolin-Enriched Membrane Preparations. CEMs were prepared as previously described (*9*) using the detergent-free method.

Site-Directed Mutagenesis. The ILK cav-binding domain was mutated using the Altered Sites II *in vitro* Mutagenesis System (Promega Corp., Madison, WI). ILK-wt was subcloned into the EcoRI site of pALTER-Ex2 and subjected to three consecutive mutagenesis reactions. The consensus sequence for the cav-binding domain of ILK, beginning at amino acid residue 376, is WSFAVLLW. The tryptophan and phenylalanine residues were mutated to alanine using three primers: CGCTCAGCAGACATG**GC**GAGTTTTG-CAGTGCTTC, GCAGACATGGCGAGT**GC**TGCAGTGCT-TCTGTGG, and GCTGCAGTGCTTCTG**GC**GGAACTG-GTGACACGG (mutagenic bases in bold type). Mutant DNA strands were then formed using the manufacturer's protocol. Hybrid DNA molecules were transformed into XLmutS competent cells (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The transformation reaction mixture was diluted into 5 mL of LB broth containing antibiotic and grown overnight at 37 °C and 225 rpm. Mutant plasmid DNA was purified using Qiagen mini-prep spin columns (Qiagen, Inc., Valencia, CA) and transformed into competent JM109 cells (Promega). ILK-mutCavbd was then subcloned into the pcDNA3.1-V5 vector and the resulting construct sequenced to ensure successful mutation.

Western Immunoblotting. Western blot analyses were performed as previously described (*10*), using OCTG buffer [containing 50 mM Tris (pH 7.5), 150 mM NaCl, 60 mM *n*-octyl D-glucopyranoside (Fisher Scientific), 0.05% SDS, 0.5% Triton X-100, and protease inhibitors] to collect cell lysates. The primary antibodies that were used were anti-ILK#550, anti-ILK clone 65.1.9 (1:1000) (Upstate Cell Signaling Solutions, Charlottesville, VA), anti-Cav pAb, anti-Cav-1-mAb (clone 2297), anti-V5 (1:1000) (Invitrogen), and anti-glyceraldehyde 3-phosphate dehydrogenase (1:1000) (Chemicon International, Inc., Temecula, CA). Secondary antibodies (goat anti-rabbit and goat anti-mouse) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Blots were developed with the Phototope-HRP Western Blot Detection Kit (Cell Signaling Technology, Inc.) according to the manufacturer's instructions and exposed to film (Hyperfilm-ECL, Amersham Biosciences Corp.). In each figure, blots are one representative of three independent experiments that were performed.

Immunofluorescence. Immunocytochemistry was performed as described previously (*11*).

Immunoprecipitation. Immunoprecipitation was performed as previously described (*12*). Cell lysates were collected in OCTG buffer for immunoprecipitations with anti-cav or in modified RIPA buffer [20 mM Tris (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 10 μg/mL leupeptin, 10 μg/mL aprotonin, and 100 μg/mL PMSF] for anti-ILK.

Affinity Chromatography. An N-terminal biotinylated CSD peptide (BCSD peptide) with the amino acid sequence DGIWKASFTTFTVTKYWFYR was dissolved in OCTG buffer with 50% DMSO and added to 400 μg of protein from SHEP cell lysates to a final concentration of 0.1 mM. An N-terminal biotinylated human parathyroid hormone peptide (BpTH) with the amino acid sequence EKSLGEADKAD-VNVLTKAKSN (Bachem, King of Prussia, PA) was the negative control. After a 1.5 h incubation at room temperature, 30 μL of Neutr-Avidin beads (Pierce Biotechnology, Inc., Rockford, IL) was added for 2 h, then washed three times in OCTG buffer, resuspended in 20 μL of 2× sample buffer, and subjected to SDS−PAGE followed by Western blotting.

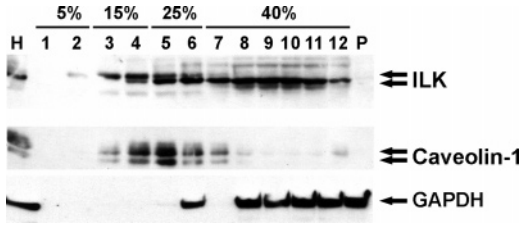

FIGURE 1: CEMs of untransfected SHEP cells. ILK is present in fractions 2−12 and the initial homogenate (H) (top blot). Caveolin-1 is enriched in low-density membranes, present in fractions 3−7 (middle blot). GAPDH is present primarily in fractions 8−12 (bottom blot). The percentage of sucrose in each fraction is indicated above the blots. The blots shown are one representative example of three separate experiments.

RESULTS

ILK Is Present within Cav-Enriched Membranes. The presence of a putative cav-binding domain within ILK suggests that cav-1 and ILK are binding partners. If ILK binds to cav-1, we would expect to detect ILK within cav-enriched membranes (CEMs). Therefore, CEMs were isolated from untransfected SHEP cell lysates using sucrose gradient density centrifugation and subsequently analyzed by Western immunoblotting. ILK is present in the original homogenate, and in fractions 3−12 relatively equally (top to bottom) (Figure 1, top blot). Cav-1 is enriched in the more buoyant CEMs around the 15−25% sucrose (fractions 3−7); as shown, a proportion of ILK is also contained within the CEM-containing fractions (Figure 1, middle blot). Interestingly, the ILK in CEM fractions 3−6 runs at a slightly higher molecular weight than the ILK in the soluble fractions. Treatment of fraction 3 with a phosphatase did not decrease the molecular weight of the ILK within fraction 3 (data not shown). The phosphatase reaction was successful, however, in decreasing the intensity of Ser473-phosphorylated Akt in SHEP cell lysates. GAPDH, which cannot be immunoprecipitated with Cav-1 as discussed below, is present primarily in fractions 8−12 (Figure 1, bottom blot). These results suggest that ILK is present in the soluble fractions as well as in the fractions containing CEMs and that the increase in the molecular weight of ILK in the CEMs is not due to phosphorylation.

ILK and Cav Expression in SHEP Human Neuroblastoma Cells. SHEP cells were transfected with pcDNA3.1 (ILK-v), wild-type ILK (ILK-wt), kinase-deficient ILK (ILK-kd), or mutant caveolin-binding domain ILK (ILK-mutCavbd) to characterize the putative interaction of ILK and caveolin-1. Figure 2A demonstrates expression of endogenous and recombinant ILK in these cells. Expression of recombinant ILK protein is detected using either a polyclonal ILK

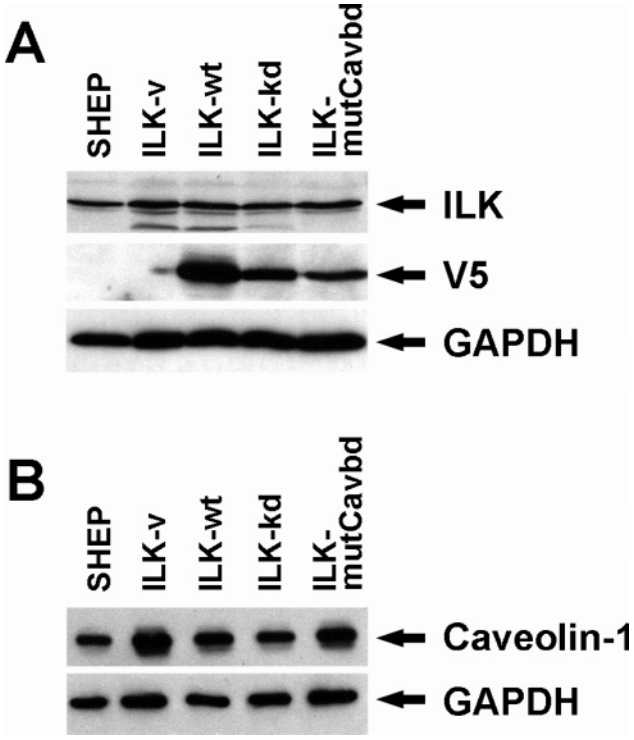

FIGURE 2: ILK and caveolin-1 expression in SHEP cells. (A) Western blot of untransfected SHEP cells. The top blot is probed with anti-ILK. The middle blot is probed with anti-V5, and the bottom blot is probed with GAPDH as a loading control. Only ILK-wt, ILK-kd, and ILK-mutCavbd express recombinant ILK. The top, middle, and bottom blots are derived from the same nitrocellulose sheet that has been stripped and reprobed. (B) Western blot with anti-caveolin pAb and anti-GAPDH as a loading control.

antibody or an antibody to the epitope tag, V5. Immunoblotting for the epitope tag shows expression of V5 only in cells expressing recombinant ILK. The polyclonal ILK antibody frequently detects more than one band; these extra bands may be splice variants of ILK, since the ILK gene is composed of 13 exons (*13*). Cav-1 is also expressed in untransfected SHEP cells and in all of the ILK transfectants (Figure 2B). Differences in cav-1 expression among SHEP cells and ILK transfectants are probably due to minor differences in the confluency of the cells, since cav-1 expression changes as cells progress through the cell cycle (*14*).

ILK and Cav-1 Colocalize in SHEP Cells. ILK and cav-1 localization was visualized by labeling ILK transfectants with ILK and cav-1 antibodies. Endogenous ILK is localized to the cytoplasm, the cytoskeleton, and the perinuclear area in ILK transfectants plated on glass coverslips (Figure 3). Cav-1 is present throughout the cytoplasm, at the edges of the cells, and also in the perinuclear area. Endogenous ILK and caveolin-1 therefore overlap especially in the perinuclear area, and occasionally at the edges of the cell (Figure 3). In ILK-wt cells, ILK staining is observed along the cytoskeleton, in the perinuclear region, and potentially in focal adhesions. In ILK-kd and ILK-mutCavbd cells, on the other hand, ILK expression is primarily dispersed throughout the cytoplasm, although in ILK-mutCavbd cells ILK appears to be more internal, away from the periphery of the cell (Figure 3). Moreover, ILK-mutCavbd cells appear to be less well-spread, especially in comparison to ILK-wt cells. These data suggest that ILK and cav-1 may colocalize within the cell and that mutation of the ILK cav-binding domain may affect cellular morphology.

Cav-1 and ILK Form a Complex in SHEP Cells. Since ILK is present within CEMS and also colocalizes with cav-1 in ILK-wt cells, we wanted to determine whether ILK and cav-1 form a complex in SHEP cells. SHEP cell lysates were immunoprecipitated with the anti-cav-1 antibody. The anti-cav antibody co-immunoprecipitates a 59 kDa protein that is immunoreactive with the anti-ILK antibody in ILK-wt and ILK-kd cells (Figure 4A, top blot). The magnitude of this band is decreased in ILK-mutCavbd cells, suggesting that the mutated cav-binding domain of ILK hinders binding of cav-1 to ILK. The decrease in the level of ILK-mutCavbd and cav-1 binding is not due to differences in protein levels, however, since the levels of the IgG heavy chain and immunoprecipitated cav-1 remain the same in all lanes (Figure 4A). These results have been quantified with densitometry and expressed as the ILK:heavy chain ratio. The ILK:heavy chain ratio in ILK-mutCavbd cells is 36.7 $\pm$ 0.071% of that of the other cell lines, reflecting a greater than 60% decrease in the extent of co-immunoprecipitation. Additionally, the immunoprecipitation of ILK by the anti-cav-1 antibody is specific since anti-cav-1 is unable to co-immunoprecipitate GAPDH (data not shown). SHEP cell lysates were also immunoprecipitated with the anti-ILK antibody, which co-immunoprecipitates a 24 kDa protein that is immunoreactive with the anti-cav-1 antibody (Figure 4B). SHEP cells have a large amount of endogenous ILK, however, therefore preventing visualization of the decrease in the level of ILK-mutCavbd binding.

To confirm that the extent of binding of ILK-mutCavbd cells to cav-1 has decreased, CEMs were isolated from these cells and analyzed via Western immunoblotting. Blots were probed with both anti-V5 to detect ILK-mtCavbd expression and cav-1 to ensure the proper fractionation and success of the immunoblotting procedure. V5 immunoblotting reveals ILK-mutCavbd in fractions 4 and 6 (Figure 4C). In contrast, cav-1 is present primarily in fractions 3−8, with additional expression detected on this overexposed film (necessary for detection of V5 in the membrane fractions) in fractions 2, 8, 9, 11, and 12 (Figure 4C). These data confirm our immunoprecipitation data, namely, that the extent of ILK-mutCavbd complex formation with cav-1 is greatly reduced in neuroblastoma cells.

The Cav-Scaffolding Domain of Cav and the Cav-Binding Domain of ILK Mediate the ILK−Cav Interaction. The immunoprecipitation studies described above suggested that the cav-binding domain of ILK is important in formation of a complex with cav. To examine this interaction more closely and to assess the involvement of the cav-scaffolding domain of cav, we assessed binding of ILK to the cav-scaffolding domain *in vitro*. We hypothesized that the cav-scaffolding domain of cav-1 and the cav-binding domain of ILK are essential in the ILK−cav-1 interaction.

An N-terminal biotinylated peptide generated to the cav-scaffolding domain of cav-1 (BCSD peptide) was added to ILK-wt or ILK-mutCavbd lysates, and the resulting lysates were applied to Neutr-Avidin beads. Additionally, an N-terminal biotinylated human parathyroid hormone peptide (BpTH) was added to some of the lysates as a negative control. Immunoblotting with the V5 antibody (Figure 5A) shows that a band of 59 kDa is affinity-purified from ILK-

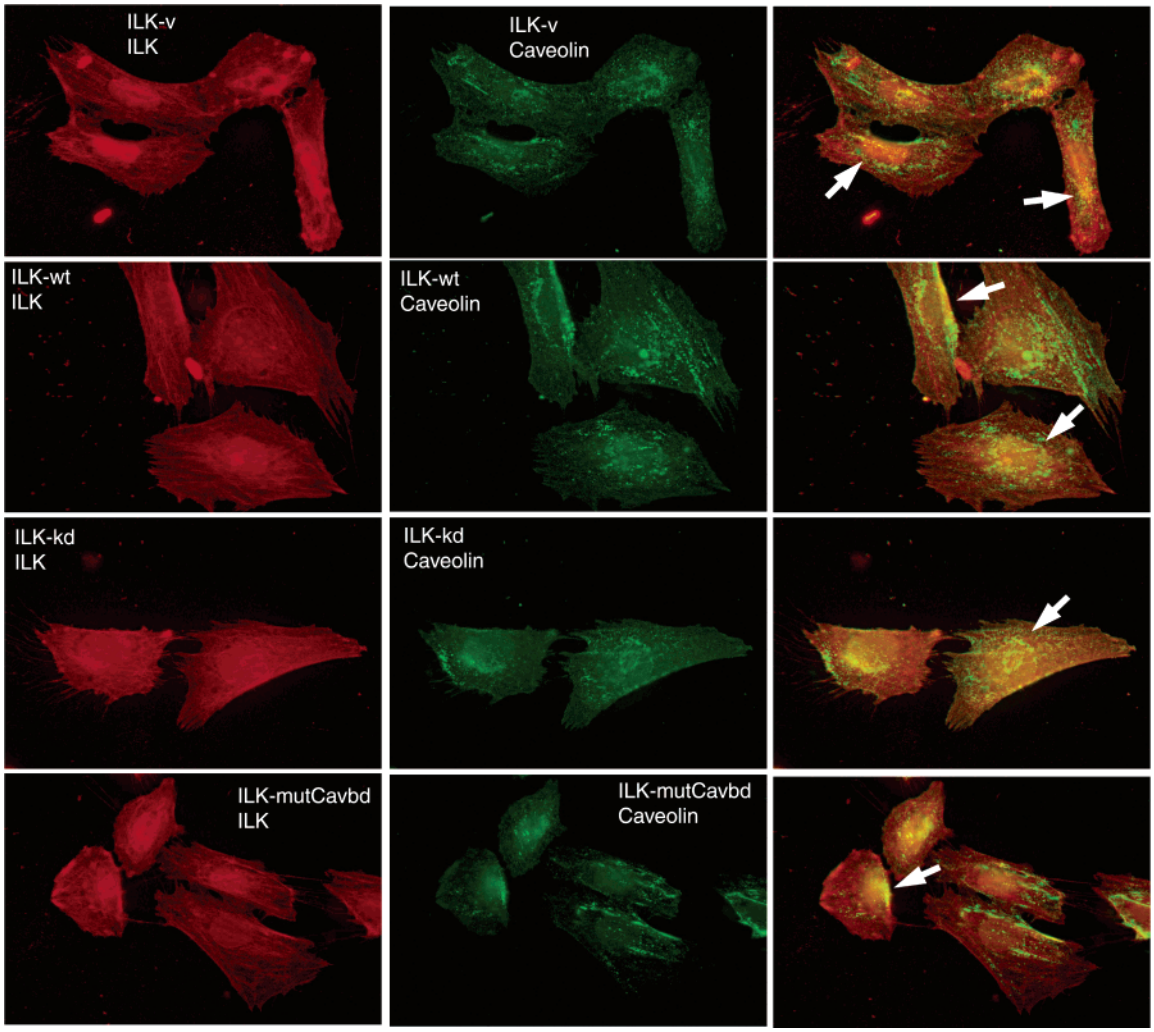

FIGURE 3: Localization of ILK and caveolin-1 in SHEP cells. Each confocal image is labeled with the cell type that is shown. Green fluorescence represents caveolin-1 expression, while red fluorescence reflects ILK expression. Yellow fluorescence indicates colocalization of caveolin-1 and ILK. The images were derived from the same experiment, which was replicated three times, and are representative examples of several fields. Arrows indicate areas of apparent colocalization.

wt cells when the BCSD peptide is present. The interaction of ILK-wt is specific to the BCSD since addition of the BpTH peptide does not precipitate ILK (Figure 5B). The 59 kDa protein is not purified from the ILK-mutCavbd lysates with the BCSD peptide. These data suggest that the ILK-mutCavbd construct is deficient in binding to cav-1 and that both the cav-binding domain of ILK and the cav-scaffolding domain of cav-1 are necessary for complex formation. When the same blot was stripped and reprobed with the anti-ILK antibody (Figure 5A, top blot), the 59 kDa protein is present in both the ILK-wt and ILK-mutCavbd lysates when the BCSD peptide is present. This indicates that complex formation is inhibited by recombinant ILK-mutCavbd and that complex formation with the endogenous ILK is operative.

DISCUSSION

Cav-1 is a structural component of caveolae and regulates biological processes via binding of the cav-1-scaffolding domain contained within cav with a cav-1-binding domain located within the binding protein (*15*). The identification of a putative cav-1-binding domain sequence within ILK led us to the hypothesis that Cav-1 and ILK bind within cells. In this study, we show that ILK and cav-1 form a complex in SHEP human NBL cells. This interaction is dependent upon the cav-binding domain of ILK and the cav-scaffolding domain of cav-1. Furthermore, this interaction of ILK and cav-1 is inhibited in cells that express ILK with a mutated cav-binding domain. These results implicate a novel mechanism potentially regulating ILK binding or activity that is dependent on cav-1.

The interactions of ILK with its binding partners demonstrate that ILK is involved in integrin-mediated regulation of cytoskeletal organization, cell spreading, and cell adhesion. ILK colocalizes with a variety of proteins present in focal adhesions, including affixin, CH-ILKBP, paxillin and actopaxin, and PINCH (*16*−*19*). Disregulation of these interactions may facilitate tumorigenesis. The presence of a putative cav-binding domain within ILK suggests that binding to cav-1 might be one mechanism of decreasing ILK kinase activity or altering ILK localization, thereby suppressing cellular transformation.

Cav-1 is a 21−24 kDa hairpin-shaped integral membrane protein (*20*). The N-terminus contains the cav-scaffolding domain (residues 82−101) that is required for homo-oligomerization and for direct binding to other signaling

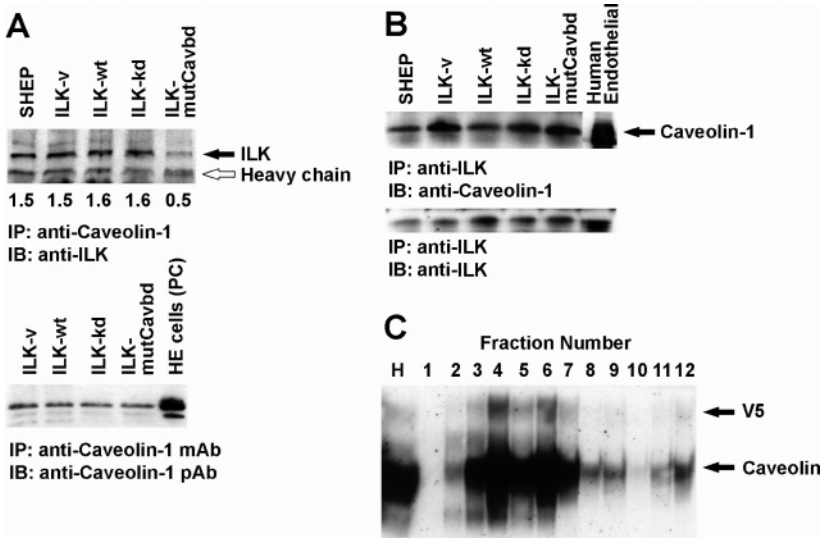

FIGURE 4: Immunoprecipitations of ILK and caveolin-1. (A) Western blot with anti-ILK after immunoprecipitation with anti-caveolin-1 monoclonal antibody (top blot). The top band (black arrow) is ILK, and the bottom band (white arrow) is the IgG heavy chain. Densitometry results are expressed as the ILK:heavy chain ratio under each respective band. The bottom blot shows immunoprecipitation of caveolin. (B) Western blot with anti-caveolin-1 after immunoprecipitation with anti-ILK (top blot). The bottom blot shows immunoprecipitation of ILK. Blots and densitometry are one representative example from three experiments. (C) CEMs for ILK-mutCavbd cells probed with anti-V5 and Cav-1. V5 is present in fractions 4 and 6 in ILK-mutCavbd cells, whereas Cav-1 is present primarily in fractions 3−7.

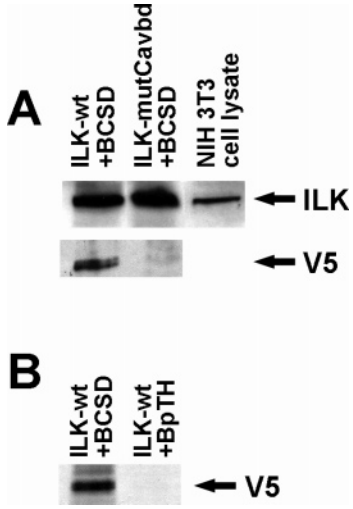

FIGURE 5: Affinity chromatography of ILK with the biotinylated caveolin-scaffolding domain peptide. (A) Western blot with anti-ILK (top blot) or anti-V5 (bottom blot) after affinity chromatography using Neutr-Avidin beads and a biotinylated caveolin-scaffolding domain peptide. The top and bottom blots are derived from the same nitrocellulose sheet that was stripped and reprobed. (B) Western blot with anti-V5 after affinity chromatography with Neutr-Avidin beads and either the BCSD peptide or the BpTH peptide. Association of recombinant ILK is specific to the BCSD peptide, since the BpTH peptide does not associate with ILK-wt. The blots shown are one representative example of three separate experiments.

molecules via the caveolin-binding domain (*15*). The consensus sequence for the cav-binding domain is ΦXΦXXXXΦ or ΦXXXXΦXXΦ, where Φ represents an aromatic amino acid. Many signaling molecules contain cav-binding domains, including G-proteins, receptor tyrosine kinases, growth factor receptors, and ILK (*21*−*24*). Many signaling proteins bound to the cav-scaffolding domain are inhibited. For example, mutational or pharmacologic blockade of G protein subunits prevents binding to cav, indicating that only the inactive GDP-bound G-protein subunits interact with cav. Cav further regulates the G-protein second-messenger system by binding to protein kinase A (*22*). Protein kinase A expression is present in punctate regions of the cell membrane in cells that also express cav-1. PKA expression is present throughout the cytoplasm, however, in cells that do not express cav-1. cAMP-mediated signaling is inhibited in cells expressing cav-1. Similarly, caveolin-1 preferentially interacts with inactive Ras and Src, as mutationally active Ras and Src do not bind to cav-1 (*23*, *24*). After the proper stimulus, the preassembled signaling molecules dissociate from cav to become active (*25*).

The existence of ILK within CEMs (fractions 3−7) suggests that ILK and cav-1 are present within the same cellular compartment (Figure 1). ILK is also present in the soluble fractions (8−12), however, indicating that two pools of ILK may be present within cells (Figure 1). The portion of ILK that is present within CEMs may be bound to and/or under regulation by cav-1, while the portion of ILK that is present within the soluble fractions may not be bound to or regulated by cav-1. Our findings are supported by T. Deng and colleagues, who report that ILK is present in two distinct populations in smooth muscle cells (*26*). A Triton X-100 insoluble component is able to phosphorylate myosin in a calcium-independent manner. As cav-1 is also Triton X-100 insoluble, the ILK present in these fractions may be associated with cav-1 (*26*).

To study the putative ILK−cav-1 interaction, we mutated the cav-binding domain of ILK-wt. Specifically, the three required aromatic amino acid residues within the cav-binding domain were mutated to alanine residues. The resulting construct, ILK-mutCavbd, was stably transfected into SHEP cells for binding and localization studies. While ILK-wt and ILK-kd colocalize with cav-1, the distribution of ILK and cav-1 changes in ILK-mutCavbd (Figure 3). Furthermore, ILK-mutCavbd cells appear rounder and less well-spread (Figure 3), consistent with a role for ILK in cytoskeletal organization, cell spreading, and cell adhesion. Besides changing the distribution of ILK and cav-1, ILK-mutCavbd binds to cav-1 at a greatly reduced level when compared

with ILK-wt and ILK-kd cells (Figures 4 and 5). These data show that the cav-binding domain of ILK and the scaffolding domain of cav-1 are necessary for complex formation. We are currently investigating whether the binding of ILK to cav-1 changes the kinase activity of ILK.

Controversy about whether ILK is a kinase or an adapter protein exists. The sequence of the C-terminal domain of ILK is homologous with those of other serine/threonine kinases, but lacks both HRDLXXN and DFG motifs that are usually required for kinase activity (*27*). Initially, the kinase activity of ILK was assayed by creating a GST fusion protein that was isolated by SDS−PAGE followed by gel extraction. The resulting protein was found to phosphorylate proteins *in vitro* (*6*). No one has shown direct kinase activity of ILK *in vivo*. Dominant negative ILK (L220M) does not demonstrate kinase activity; thus, the mutated residue might be important for kinase activity, or it might be critical for binding to an as yet unidentified kinase. Lynch and colleagues further mutated L220M-ILK, changing a critical serine residue to alanine, aspartate, or glutamate. Dominant negative ILK with the serine to alanine mutation was still deficient in kinase activity. However, mutation to aspartate or glutamate partially restored kinase activity to that of the dominant negative ILK. These data suggest that ILK may be an adapter protein that recruits other protein kinases.

Experimental evidence supports the view that overexpression or activation of wild-type ILK causes cells to express a more tumorigenic and less differentiated phenotype. Since signaling molecules that bind to cav-1 are usually bound in an inactive state, we propose a hypothesis in which inactive, wild-type ILK is bound to cav-1 and sequestered at the cell membrane. Upon integrin-mediated or growth factor receptor-mediated signaling events, ILK dissociates from cav-1 and exerts cellular effects. Cells that lack cav-1 or overexpress ILK may be especially at risk for transformation, since excess ILK would be free to increase the rate of cell survival and a transformed phenotype. Therefore, the interaction between ILK and cav-1 may be a useful target for genetic screening procedures of human neuroblastoma. Given the roles of cav-1 and ILK in the regulation of cell growth, survival, and transformation, dysregulation of their interaction is likely to have wider implications in many forms of cancer.

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