Filamin and filamin-binding proteins in integrin-regulation and adhesion. Focus on: “FilaminA is required for vimentin-mediated cell adhesion and spreading”

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Integrin-mediated cell adhesion is a fundamental process in cell biology. The heterodimeric integrin transmembrane proteins play a key role in physiological processes such as embryogenesis, wound healing, and immune defense but also in pathological processes such as cancer and autoimmune disease. Thus research investigating the function and regulation of integrins is of fundamental importance, and several major discoveries have been made recently. Novel integrin regulators talin and kindlin have been identified (11, 13), and the mechanisms through which they regulate integrins are beginning to be understood (2).

Integrins exist as heterodimers consisting of α- and β-chains in different combinations, forming at least 24 subtypes. They have large extracellular domains that bind ligands in the extracellular matrix or on the surface of other cells, and short intracellular domains that interact with cytoplasmic proteins. Integrins are not constitutively active, but instead bind ligands only after so-called “activation” or “inside-out signaling”, the final step of which appears to be the binding of cytoplasmic proteins such as talin, kindlin, 14–3-3 proteins, and perhaps others to the integrin cytoplasmic domains (2, 10). This results in changes in integrin conformation/affinity for ligands, as well as the clustering of integrins on the cell surface, leading to an overall increase in cell adhesion. After ligand binding, the interaction of integrins with the actin cytoskeleton and other proteins induces further changes in cell behavior (so called “outside-in signaling”), including cell spreading and actin reorganization. Furthermore, integrins are also continuously recycled within the cell during processes such as cell migration, adding yet more complexity to the regulation of these molecules and cell adhesion.

Filamin is a large dimeric actin binding protein whose primary function is to crosslink F-actin fibers. It also coordinates the interaction between transmembrane cell receptors (including integrins) and the cytoskeleton (15). Filamin consists of an NH2-terminal actin binding domain and COOH-terminal dimerization domain flanking 24 tandem immunoglobulin-like domains that mediate protein interactions. This allows filamin to act as a molecular scaffold, both tethering membrane receptors (such as epidermal growth factor receptor, insulin receptor, and glycoprotein Ibα) to the actin cytoskeleton, and colocalizing signaling molecules involved in related signal transduction(1, 4, 16). Most interacting receptor molecules bind to the immunoglobulin-like domains at a conserved structural region, where binding is coordinated through hydro-
gen bonding and hydrophobic contacts within an array of β-strands (7, 12, 17).

Filamin plays a major role in actin cytoskeletal rearrangement. It has been demonstrated to bind to the rac-specific GTPase binding protein FilGAP targeting this molecule to sites of membrane protrusion where it is involved in lamellipodia formation. Furthermore, cell spreading is perturbed by disruption of this interaction through mutations at the FilGAP binding site in filamin (12, 14). Also, filamin interacts with p21-activated (Pak1) at membrane ruffles, and this interaction is necessary for membrane ruffle formation and cytoskeletal rearrangement, independent of GTPase activity (18). Filamin has also long been known to interact with integrin cytoplasmic tails thus mediating integrin-actin interactions. Integrin activation also long been known to interact with integrin cytoplasmic tails during integrin-actin interactions. Integrin activation appears to be mediated through competition between integrin regulators such as filamin, talin, and 14–3–3 proteins. Here, filamin appears to stabilize the inactive/low affinity conformation, since disruption of its association with integrin by the cytoskeletal adapter protein migfilin leads to an increase in β3 integrin activation (5). Furthermore, knockdown of filamin leads to an increase in β1 and β2 integrin activation and cellular adhesion (7, 17). However, filamin has also been reported to play a role in integrin-mediated cell spreading and in mechanoresponses mediated by integrins, thus mediating integrin outside-in signaling (3, 9).

In a recent study by Kim et al. (8), the interplay between filamin and the intermediate filament protein vimentin and the role of these proteins in β1 integrin surface expression, adhesion, and spreading on collagen has been examined. A novel interaction between filamin and vimentin is described, discovered through isotope-coded affinity tag analysis of filamin immunoprecipitates from cells spreading on collagen, and confirmed by coimmunoprecipitation and coimmunofluorescence analysis. The interaction appears to be direct, as determined by pulldown assays with purified components. Both small interfering RNA (siRNA) knockdown of filamin and of vimentin reduced cell spreading on collagen, as well as β1-integrin surface expression, implying a role for both proteins in integrin trafficking. It had been previously reported that protein kinase C (PKC) phosphorylation of vimentin regulates recycling of endocytosed integrins to the plasma membrane (6). Here, Kim et al. provide evidence that this interaction may be mediated through a shared association with filamin A, further demonstrating that in the absence of filamin A, PKC-ε-specific phosphorylation of vimentin does not take place, thus compromising cell spreading and adhesion. A model is proposed where these three components regulate integrin trafficking and possibly activation, as well as demonstrating the pivotal role of filamin in linking integrins to intermediate filaments and the actin cytoskeleton (see Fig. 10 in Ref. 8).

The study by Kim et al. further emphasizes the complex role of filamin as a signaling scaffold in the regulation of integrins and cell adhesion. Filamin appears to play a dual role in integrin regulation, being involved in both inhibition of integrin activation (through direct binding to integrin tails) and the trafficking of integrins to the plasma membrane and subsequently cell spreading (through the interaction with other molecules, such as vimentin and PKC). In the study by Kim et al., a large effect of filamin knockdown on integrin surface expression is observed, implying a role for filamin/vimentin in integrin trafficking. This may explain the different experimental outcomes with regard to integrin-mediated cell adhesion when interfering with the function/expression of filamin (5, 7, 17). In other studies, it has been reported that knocking down filamin leads to an increase in integrin activation and cell adhesion, whereas Kim et al. report the opposite. If there are less integrins on the cell surface, as described in the current study, it is understandable that adhesion and further downstream events (cell spreading) are reduced as well. Other explanations for the differential outcomes could be the cell type used or the integrin subtype involved.

The paper by Kim et al. makes an interesting contribution to the understanding of the function of this extremely versatile scaffolding molecule, introducing yet another interaction partner for filamin (Fig. 1). This extends the functions of filamin to perhaps also being an integrator of the actin cytoskeleton and intermediate filament systems.

Because filamin is such a complex scaffolding molecule and has so many interacting partners, it is problematic to study. Are there different pools of filamin in cells, interacting with different partners and thus performing different roles at different times and in different cell types? To further clarify the role of filamin/vimentin in cell adhesion, we await more sophisticated tools to efficiently separate the proposed functions of filaminA in integrin regulation/cell spreading, such as genetic models where specific filamin interactions are disrupted, and in vivo/in situ imaging techniques that can follow these interactions in real time.

GRANTS

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DISCLOSURES

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REFERENCES