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The weakest link

A new paradigm for stabilizing the integrin–actin connection

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The extracellular matrix (ECM) is a protein scaffold that is assembled by cells into a precise configuration and constantly remodeled. It not only provides structural support for cells and tissues, but also provides positional cues for cell adhesion and migration and serves as a reservoir for growth factors. Consequently, organ development and homeostasis critically depend on cell–ECM interactions. On the other hand, disturbances in ECM deposition and remodeling contribute to organ dysfunction and disease, such as fibrosis.[1] The interaction of cells with the ECM occurs mainly through integrins, heterodimeric transmembrane receptors for ECM components. Their central function is to link the ECM to the actin cytoskeleton to enable cellular force transduction. This is important for regulation of cell shape, migration, and ECM remodeling. Integrins themselves lack actin-binding properties, so they recruit a large number of scaffold proteins, kinases, and other regulatory proteins to engage and remodel the cytoskeleton. These large, multi-protein complexes, termed focal adhesions (FA), are essential for integrin function.[2]

Integrin-linked kinase (ILK) is an integrin-binding protein that plays an essential role in the establishment and maintenance of the integrin–actin connection. However, the exact function of this protein has long remained unclear and controversial due to its various reported catalytic and scaffolding properties. As its name implies, ILK shows high sequence homology to kinases and also folds like a typical protein kinase, but it lacks several conserved motifs present in eukaryotic protein kinases and has been recently shown to lack catalytic activity.[3-5] Interestingly, the pseudo-active catalytic site of ILK binds another adaptor, termed parvin, in a manner resembling a kinase–substrate interaction.[4] Parvins exist in 3 isoforms in vertebrates and are characterized by 2 in-tandem arranged calponin homology (CH) domains that constitute an actin-binding domain. The second CH domain also mediates the interaction with ILK and targets the complex to FAs, providing a direct link between integrins and the actin cytoskeleton. Consequently, ILK-deficient fibroblasts display a severe delay in the formation of FAs. Once established, the FAs are smaller in size and poorly linked to a disorganized actin cytoskeleton, highlighting the importance of ILK in regulating actin engagement downstream of integrins.[3]

The integrin–actin linkage, although mechanically stable, is a network of highly dynamic interactions between the various FA components and F-actin. This facilitates the connection of the relatively static, ECM-bound integrins to the constantly treadmilling F-actin network, particularly during cell motility. How this dynamics is achieved and spatiotemporally linked to adhesion turnover is still incompletely understood. In our recent study, we aimed to understand how the turnover of ILK is regulated.[6] Biochemical analyses of posttranslational modifications on ILK revealed that it is robustly ubiquitinated and carries both lysine 48- and 63-linked ubiquitin chains. In a subsequent proteomic interaction screen, we identified the E3 ligase CHIP and the chaperone heat shock protein 90 (Hsp90) as novel interactors of ILK. Hsp90 was found to stabilize ILK, facilitating the interaction of ILK with parvin. When Hsp90 activity was blocked, ILK was polyubiquitinated by CHIP and degraded by the proteasome (**Fig. 1**).[6]

We propose that the kinase fold of ILK provides the structural basis for its recognition by Hsp90, a chaperone that specifically recognizes and assists the folding of so-called client proteins that are mostly kinases.[7] Interestingly, previous proteomic studies have indicated Hsp90 to be a component of cell–matrix interactions.[8] The role of Hsp90 and other chaperones at FAs has, however, remained elusive. In line with the proteomic studies, we found Hsp90 to co-localize with ILK in large peripheral FAs. Given that these sites are subject to high local traction forces, it is possible that the inherently instable kinase domain of ILK might represent a weak structural link susceptible for force-induced unfolding. The presence of Hsp90 at FAs might be required to stabilize the correct fold of the kinase domain and to maintain the critical interaction of ILK with parvin. This would explain the observed rapid removal of ILK from large peripheral FAs upon inhibition of Hsp90 activity and the subsequent loss of force-bearing adhesions in these cells.

A stable integrin–actin linkage and cellular force generation are prerequisites both for productive cell migration and ECM remodeling. Intriguingly we found that inhibition of Hsp90 activity leads to dramatic attenuation of both fibroblast motility and fibronectin matrix deposition. This prompted us to assess whether inhibition of Hsp90 might be a potent means to block fibrosis, pathological

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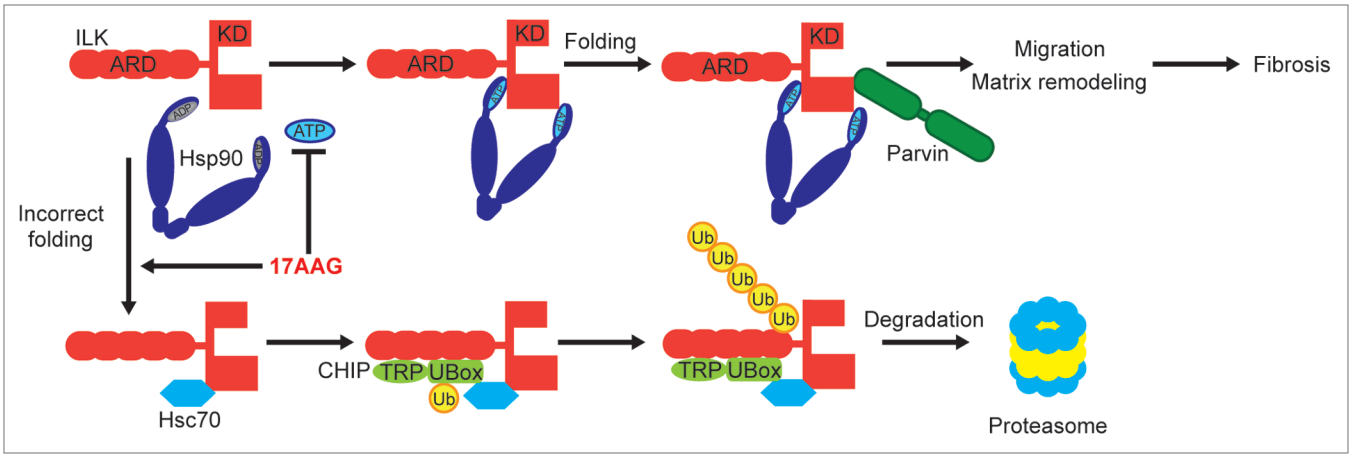

Figure 1. A model of how stability of ILK is regulated by the Hsp90/CHIP axis. Binding of Hsp90 to the kinase domain (KD) of ILK stabilizes ILK and enables its interaction with parvin. The ILK–parvin complex links integrins to the actin cytoskeleton at focal adhesion sites and facilitates cellular force generation, a prerequisite for migration and matrix remodeling. Incorrect folding of ILK or blocking of Hsp90 activity by 17AAG leads to the recruitment of the chaperone Hsc70 and E3 ligase CHIP to bind ILK. CHIP binds via its UBox domain to the ankyrin-repeat domain (ARD) of ILK, resulting in polyubiquitination of ILK and its subsequent targeting for proteasomal degradation. Pathological deposition of extracellular matrix in fibrosis can be attenuated by inhibiting Hsp90.

accumulation of ECM in tissues. Indeed, in a mouse model of bleomycin-induced fibrosis, inhibition of Hsp90 could completely block the fibrotic response.[6] It has previously been shown that targeting Hsp90 is an effective way to treat certain cancers as many key oncogenes are clients of this chaperone.[7] Our study implicates that this strategy could also be successful in the treatment of fibrosis, a life-threatening disease lacking effective therapy. In addition to ILK, other central FA regulators such as the Src family kinases and focal adhesion kinase have been shown to be clients of Hsp90,[7] likely explaining the efficacy of Hsp90 inhibition in blocking fibrosis in the mouse and providing interesting insights to the role of chaperones at cell adhesion sites.

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