Integrin-linked kinase: a Scaffold protein unique among its ilk

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Abstract Integrin-linked kinase (ILK) is a scaffolding protein with central roles in tissue development and homeostasis. Much debate has focused on whether ILK is a *bona fide* or a pseudo-kinase. This aspect of ILK function has been complicated by the large volumes of conflicting observations obtained from a wide variety of experimental approaches, from *in vitro* models, to analyses in invertebrates and in mammals. Key findings in support or against the notion that ILK is catalytically active are summarized. The importance of ILK as an adaptor protein is well established, and defining its role as a signaling hub will be the next key step to understand its distinct biological roles across tissues and species.

Keywords Integrin-linked kinase · α -parvin · Akt · Scaffold proteins · Kinase

Abbreviations

ECM Extracellular matrix
GST Glutathione-S-transferase
ILK Integrin-linked kinase
PH Pleckstrin homology

The interactions between cells and their surrounding ECM play key roles in normal tissue homeostasis, and their

disruption is involved in the pathogenesis of numerous human disorders. Cell adhesion to the ECM is mediated by integrins (Hynes, 2002) and other transmembrane proteins. Unlike many other types of receptors, integrins have no intrinsic signaling or catalytic properties. Cellular responses to ECM stimuli require the recruitment to sites of integrin clustering of cytoplasmic adaptor proteins. The latter, together with integrins, form multiprotein complexes termed focal adhesions, which allow the initiation of a variety of signaling cascades (Legate et al., 2009). In particular, the association of integrins with ILK regulates important aspects of cell function, including attachment to the ECM, acquisition of front-rear polarity, migration, survival and transformation (McDonald et al., 2008). The importance of ILK is abundantly clear, as it is indispensable for the development and/or function of every tissue examined to-date, ranging from bone and cartilage, to cardiovascular, hematopoietic and nervous systems, liver, kidney, skeletal muscle and skin (Wickstrom et al., 2010). ILK was identified over a decade ago, and was widely recognized as a *bona fide* kinase for several years, until studies in invertebrate and mammalian systems indicated that structural alterations that degraded its catalytic properties had no effect on its biological activity (Mackinnon et al., 2002; Sakai et al., 2003; Zervas et al., 2001). From those key reports, ILK was defined as an important hub for protein-protein interactions, rather than a kinase, triggering a flurry of efforts that have continued for a decade, focused on addressing this contention. As a result, and aside from the considerable volume of conflicting information that has been generated, the notion that ILK plays important scaffold roles in multiple tissues and organs has been reinforced.

ILK was identified as an integrin β 1-interacting protein in a yeast two-hybrid screen (Hannigan et al., 1996). In

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silico analysis of its amino acid sequence indicated the existence of 4 putative ankyrin repeats at its N-terminus, a central PH domain, and a C-terminal region with high, but imperfect homology to *bona fide* kinase domains. However, significant departures from the *in silico* predictions have become apparent from crystallographic studies, resulting in the identification of not four, but five ankyrin repeats, and the observation that the predicted PH motif may not functionally exist, as this region cannot bind PIP₃ (Chiswell et al., 2010; Fukuda et al., 2009). Given the importance of ILK, a substantial body of work has focused on two aspects that to-date remain highly controversial: The kinase properties of ILK, and its potential as a general therapeutic target.

Structural analyses of full-length ILK have yet to be reported. However, detailed crystallographic studies of the ILK C-terminal putative kinase domain (amino acid residues 183–452) bound to the CH2 region of α -parvin have been conducted (Fukuda et al., 2009). In this complex, the ILK kinase domain exhibits an overall structure that strongly resembles that of other kinases, including its ability to bind ATP. However, under these conditions, the kinase domain does not catalyze ATP hydrolysis, which is an essential step for kinase function. It has been subsequently suggested that the lack of ILK kinase activity in α -parvin-containing complexes may arise from the fact that the latter acts as a negative regulator of ILK (Maydan et al., 2010). However, this observation contrasts with previous reports that α -parvin interactions with ILK actually stimulate its activity, at least in the context of ILK immunoprecipitates isolated from DU145 cells (Attwell et al., 2003). Other important features that diverge in the ILK kinase domain include the deletion or substitution of residues that are key for catalytic activity in other kinases (Fukuda et al., 2009). Thus, although sequence-based analyses predict reasonable homology of the ILK kinase C-terminus with other kinases, 3D structural characterization indicates that the divergence is important enough to preclude catalytic activity, at least when bound to the α -parvin CH2 domain.

In vitro assays to examine the kinase properties of ILK have also yielded conflicting results. Thus, whereas a bacterially produced GST-ILK fusion protein purified by denaturing polyacrylamide gel electrophoresis was reported to phosphorylate myelin basic protein (Hannigan et al., 1996), neither full-length bacterially produced ILK purified from a complex containing the LIM 1–2 domain of PINCH, nor recombinant, commercial ILK exhibited any measurable kinase activity (Fukuda et al., 2009). More recently, studies with baculovirus-produced ILK isolated to about 94% purity showed *in vitro* phosphorylation of a GSK-3 β substrate peptide, of LC₂₀ and of ILK itself, although no information regarding Akt, another putative ILK substrate, was reported (Maydan et al., 2010). Kinase activity in these

assays was greater in the presence of Mn²⁺ relative to Mg²⁺. The reasons why these approaches show ILK kinase activity in some cases and not in others remain undefined. It is possible that the isolation procedures for the bacterially purified proteins resulted in their denaturation and loss of activity. Given that bacterially purified ILK was originally reported to have kinase properties, the need for post-translational modifications would appear not to play a substantial role. On the other hand, it is also possible that even low levels of contaminating proteins in the baculovirus preparations may have contributed to the catalytic activity reported.

A vast number of investigators have isolated ILK immunoprecipitates from a variety of vertebrate cells and tissues, and demonstrated the presence of kinase activity in these multiprotein complexes (Attwell et al., 2003; Deng et al., 2001a; Lange et al., 2009). These studies have focused on both endogenous and exogenously expressed ILK, and have reported that ILK-containing immune complexes have the capacity to phosphorylate various physiological substrates, including Akt, GSK-3 β , LC₂₀, and myosin phosphatase (Delcommenne et al., 1998; Deng et al., 2001b; Kiss et al., 2002; Muranyi et al., 2002). In spite of the compelling evidence in support of the presence of kinase activities in ILK-containing complexes, the role of ILK itself in Akt phosphorylation remains unclear. Thus, whereas ILK inactivation by genetic or pharmacological means reportedly results in reduced Akt phosphorylation in a variety of cell lines and tissues, such as IEC-18 intestinal epithelial cells, HEK 293 human embryonic kidney cells (Delcommenne et al., 1998), in Schwann cells of the peripheral nervous system (Pereira et al., 2009), and in cardiomyocytes (White et al., 2006), similar interventions do not affect Akt phosphorylation in immortalized kidney fibroblasts (Sakai et al., 2003), chondrocytes (Grashoff et al., 2003) or primary epidermal keratinocytes (Lorenz et al., 2007). Given the scaffolding properties of ILK and the variety of proteins with which it interacts, the presence of kinase activity in ILK immune complexes is not unexpected. However, based on current evidence, it would appear that the modulating role of ILK-containing species vis-à-vis Akt may be cell- and/or context-dependent.

The consequences of disrupting residues considered key for ILK kinase function have been recently explored *in vivo* (Lange et al., 2009), reiterating the complexity of ILK and the fact that, in spite of its generalized role in actin cytoskeletal dynamics and cell adhesion, it also fulfills tissue-specific functions. Specifically, mutation of K220 to either A or M, which abrogates interactions with α -parvin, resulted in embryonic or perinatal lethality due to impaired kidney morphogenesis. These defects were attributed to alterations in actin dynamics and cell interactions with the

ECM. On the basis of the similar phenotype observed in α -parvin-deficient mice, it was proposed that the altered kidney development in mice expressing ILK K220A or K220M was likely caused by altered α -parvin localization and function, rather than by the loss of ILK kinase activity. Additional studies showing that inactivation of α -parvin in a mouse background expressing ILK K220A result in the same phenotypic alterations as those observed in the single mutants would strengthen this hypothesis, as alterations independent of α -parvin function may also be involved. Significantly, although the K220A mutation had been previously reported to abolish kinase activity (Filipenko et al., 2005; Yamaji et al., 2001), *in vitro* measurements of baculovirus-produced ILK K220A reported alterations in the kinetics of GSK-3 β peptide phosphorylation, reducing the $V_{\max}$ for this reaction to about 40% of the wild type protein (Maydan et al., 2010). The reasons for these inconsistencies are unclear. However, if the altered nephrogenesis in mice expressing ILK K220A involves reduced kinase activity, this would imply the existence of important, as yet uncharacterized dosage effects, in addition to the established role of ILK scaffolds in this tissue.

In spite of the large volume of conflicting information concerning whether and/or where ILK functions as a kinase, it is clear that ILK fulfills key functions through its scaffolding properties, albeit in tissue- and/or context-specific manner. The existence of kinase activity associated with ILK immune complexes isolated from a variety of sources has been widely demonstrated. Establishing how the scaffolding properties of ILK contribute to the recruitment of other signaling proteins, and defining the full biological significance of these signaling platforms will be a critical area for future research.

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