Integrin-linked kinase – essential roles in physiology and cancer biology

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Summary

Integrin-linked kinase (ILK) is a multifunctional intracellular effector of cell-matrix interactions and regulates many cellular processes, including growth, proliferation, survival, differentiation, migration, invasion and angiogenesis. The use of recently developed Cre-*lox*-driven recombination and RNA-interference technologies has enabled the evaluation of the physiological roles of ILK in several major organ systems. Significant developmental and tissue-homeostasis defects occur when the gene that encodes ILK is deleted, whereas the expression of ILK is often elevated in human malignancies. Although the cause(s) of ILK overexpression remain to be fully elucidated, accumulating evidence suggests that its oncogenic capacity derives from its regulation of several downstream targets that provide cells with signals that promote proliferation, survival and migration, supporting the concept that ILK is a relevant therapeutic target in human cancer. Furthermore, a global analysis of the ILK 'interactome' has yielded several novel interactions, and has revealed exciting and unexpected cellular functions of ILK that might have important implications for the development of effective therapeutic agents.

Key words: Cancer, Development, Extracellular matrix, Integrin-linked kinase, Protein-protein interaction, Signal transduction

Introduction

Integrin-linked kinase (ILK) was discovered in 1996 in a yeast two-hybrid screen using the cytoplasmic tail of 1 integrin as bait (Hannigan et al., 1996). It is a widely expressed serine/threonine protein kinase located in focal adhesions (FAs). These adhesion sites are major intracellular signaling centers, and ILK plays a central role in transducing many of the biochemical signals that are initiated by cell-matrix interactions and that regulate fundamental processes such as growth, proliferation, survival, differentiation, migration, invasion and angiogenesis (Hannigan et al., 2005; Legate et al., 2006). Evidence now points to ILK as having crucial, multifaceted roles in the normal development and function of several tissues. Furthermore, it is becoming increasingly clear that the precise cellular processes regulated by ILK are dependent on contextual cues. The tight regulation of these cellular functions is essential for tissue homeostasis, and their dysregulation is important for the development and progression of cancer. The overexpression of ILK is often a prominent feature of human malignancies and its increased abundance in tumor tissues correlates with poor patient outcome. Thus, ILK is an attractive therapeutic target in human cancer.

In this Commentary, we focus on recent advances in the understanding of physiological and pathobiological functions of ILK, which have been made possible through the use of genetic, biochemical and pharmacologic approaches. The development of therapeutic modalities targeting ILK in cancer is also explored. Finally, the discovery of new ILK-interacting proteins and the elucidation of novel functions of ILK are also highlighted.

Domain structures and interactions of ILK

ILK has a tripartite structure that underpins its multifunctional capacity (Fig. 1) – it comprises an N-terminal domain that contains four ankyrin repeats, a central pleckstrin homology (PH)-like domain and a C-terminal kinase domain. The N-terminal ankyrin repeats of ILK bind to PINCH (particularly interesting new cysteine-histidine protein, also known as LIMS1) – an adaptor protein composed entirely of LIM domains that complexes with ILK in the cytoplasm prior to active recruitment of ILK to FA sites (Zhang et al., 2002) – and to ILK-associated protein (ILKAP) – a protein phosphatase 2C (PP2C)-family protein phosphatase that negatively regulates ILK signaling (Kumar et al., 2004; Leung-Hagesteijn et al., 2001). A PH-like domain in the central region binds to phosphoinositide lipids (Hannigan et al., 2005). In addition to its catalytic function, the C-terminal domain also interacts with integrins, as well as with the FA proteins paxillin (Nikolopoulos and Turner, 2001; Nikolopoulos and Turner, 2002) and - and - parvins (Hannigan et al., 2005; Hannigan et al., 2007; Legate et al., 2006), which link ILK, and therefore integrins, to the actin cytoskeleton.

ILK and cell signaling

ILK is central to the regulation of cell signal transduction and functions as a hub around which several signaling pathways are centered (Fig. 2). Much of the work pertaining to the role of ILK in cell signaling has been accomplished using transformed and/or tumorigenic cells and it should be noted that the pathways controlled by ILK in non-transformed cells might differ from those identified in cancer cells. The kinase activity of ILK is stimulated by integrins and soluble mediators, including growth factors and chemokines (Delcommenne et al., 1998; Imanishi et al., 2007; Li et al., 2003; Rosano et al., 2006; Xie et al., 2004), and is regulated in a phosphoinositide 3-kinase (PI3K)-dependent manner (Delcommenne et al., 1998). ILK expression is also upregulated by hypoxia (Abboud et al., 2007; Lee et al., 2006). The activity of ILK is antagonized by phosphatases such as ILKAP (Kumar et al., 2004) and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (Persad et al., 2001). Importantly, early work on ILK

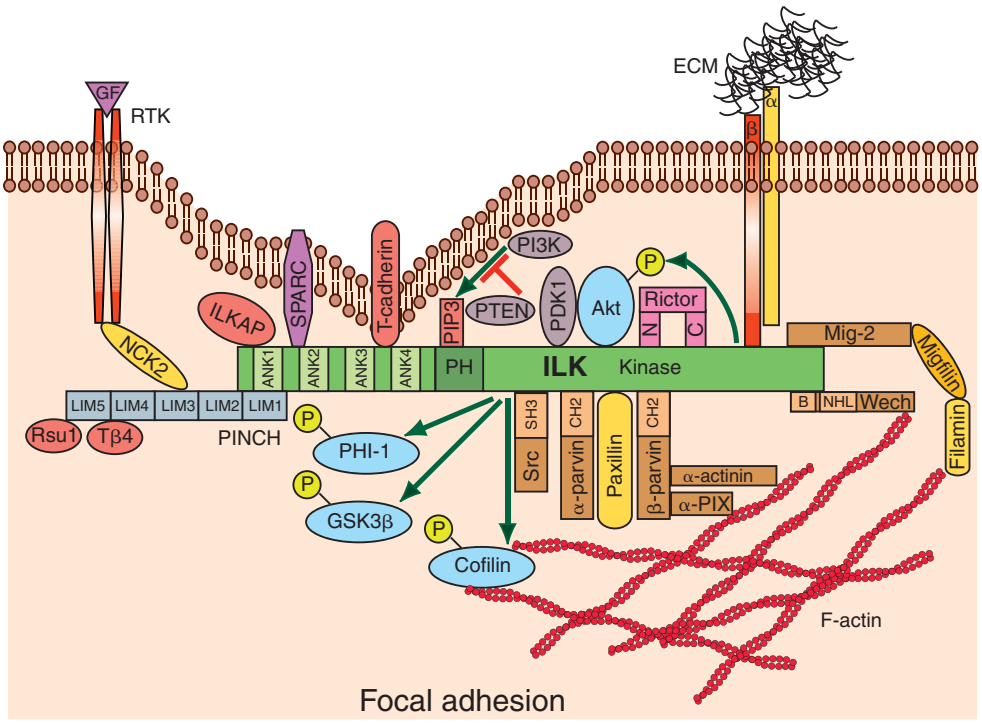

Fig. 1. The ILK interactome. ILK is localized in FAs, where it forms multiprotein complexes with several proteins that are involved in cytoskeletal dynamics and cell-signaling cascades. The N-terminal ankyrin repeats of ILK interact directly with several key proteins, including PINCH, ILKAP, SPARC and T-cadherin. PINCH also couples ILK to T 4, Rsu1 and NCK2. NCK2 associates with growth factor receptors, which potentially links ILK to growth-factor signaling. The central PH-like domain of ILK binds to PtdIns(3,4,5)P_3 and is required for PI3K-dependent activation of ILK. The C-terminal kinase domain of ILK interacts with 1 and 3 integrin, as well as with several actin-binding adaptor proteins, including -parvin, -parvin, paxillin, Mig-2 and Wech. Key binding partners that are involved in cell signaling also bind the ILK kinase domain, including PDK1, Akt, Rictor and Src. In particular, a direct interaction between Rictor and ILK is important for phosphorylation of Akt at Ser473. Rsu1, Ras suppressor 1; T 4, thymosin 4; RTK, receptor tyrosine kinase; GF, growth factor; SPARC, secreted protein acidic and rich in cysteine; PIP3, PtdIns(3,4,5)P_3; PDK1, protein-dependent kinase 1; N, N terminal; C, C terminal; P, phosphorylation site; ECM, extracellular matrix; SH3, Src homology 3; CH2, calponin homology 2; ANK, ankyrin repeat; Mig-2, mitogen-induced gene 2 (also known as fermitin family homolog 2); PHI-1, phosphatase holoenzyme inhibitor 1.

suggested that it could bind to phosphoinositide lipids via its central PH-like domain (Delcommenne et al., 1998), and a recent proteomics strategy has now validated the direct and specific binding of ILK to phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] (Pasquali et al., 2007).

After its activation, ILK exerts control over a diverse set of downstream effectors that modulate crucial cellular functions (Fig. 2). In particular, ILK has been shown to regulate the phosphorylation of Akt at Ser473 and of glycogen synthase kinase 3 (GSK3) in various cell types (Delcommenne et al., 1998; Guo et al., 2007; Imanishi et al., 2007; Joshi et al., 2007; Li et al., 2003; McDonald et al., 2008; Mills et al., 2003; Naska et al., 2006; Rosano et al., 2006; Troussard et al., 2006; White et al., 2006; Xie et al., 2004; Zhou et al., 2004). Phosphorylation of Akt at Ser473 is required for its full activation (Dillon et al., 2007). By promoting the phosphorylation of Akt, ILK stimulates signaling pathways that regulate cell survival, including those that involve caspase activation and the stimulation of nuclear factor B (NF-κB) (Hannigan et al., 2005; Legate et al., 2006). Our laboratory recently showed that siRNA-mediated depletion of ILK and Rictor, a defining member of mammalian target of rapamycin complex 2 [mTORC2 – a complex that is implicated in the phosphorylation of Akt at Ser473 (Sarbassov et al., 2005)], in breast and prostate cancer cell lines resulted in the inhibition of Akt phosphorylation at Ser473 and the induction of apoptosis (McDonald et al., 2008). Furthermore, Rictor and ILK were found to interact directly and depletion of Rictor decreased the amount of Ser473(*P*)-Akt in the ILK complex; these data suggest that Rictor facilitates the phosphorylation of Akt by ILK (McDonald et al., 2008). The phosphorylation of GSK3 by ILK regulates pathways that lead to the activation of activator protein 1 (AP1) and -catenin–LEF transcription factors, which in turn stimulate matrix metalloprotease 9 (MMP9) and cyclin D1, respectively (Hannigan et al., 2005). ILK also phosphorylates NAC, a transcriptional co-activator of AP1 (Quelo et al., 2004). Finally, ILK can modulate cell spreading, migration and cytoskeletal organization by activating PAK-interactive exchange factor (-PIX, also known as ARHGEF6), a guanine-nucleotide exchange factor (GEF) for Rac1 and Cdc42 (Filipenko et al., 2005), and by activating cofilin through an interaction with phosphorylated Src (Kim et al., 2008). High levels of ILK expression suppress expression of E-cadherin by stimulating the expression of its repressor, Snail, thereby promoting epithelial-to-mesenchymal transition (EMT) and leading to invasion and metastasis (Barbera et al., 2004).

The role of ILK in development and normal tissue physiology

Investigative strategies that involve the organism-wide loss of ILK expression and activity have demonstrated that ILK function is necessary for eukaryotic development. In invertebrates, genetic ablation experiments (Mackinnon et al., 2002; Zervas et al., 2001)

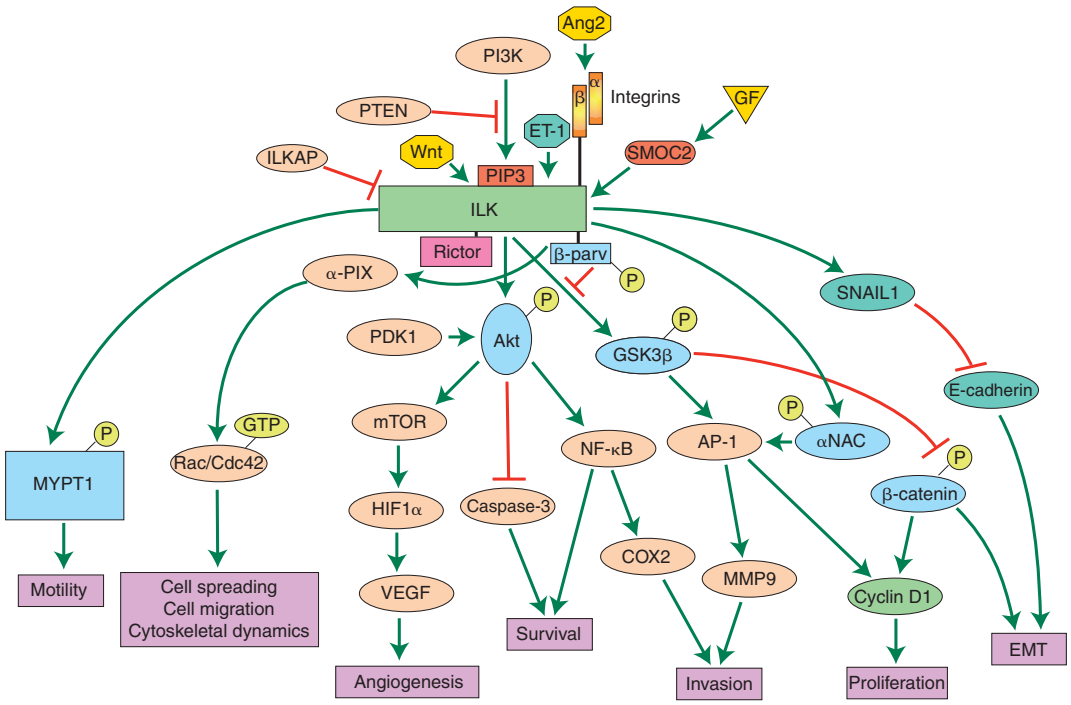

Fig. 2. Overview of the intracellular signaling pathways regulated by ILK. ILK is a central component of signaling cascades that control an array of biological processes that are crucial both to normal tissue homeostasis and to the progression of malignant disease. Activation of ILK by integrins and soluble mediators results in the regulation of downstream effectors that, in turn, modulate processes such as motility and contractility, survival, EMT, invasion, proliferation, and angiogenesis. ILK activity is antagonized by ILKAP and PTEN. Ang2, angiopoietin 2; GF, growth factor; SPARC, secreted protein acidic and rich in cysteine; PIP3, PtdIns(3,4,5)P_3; PDK1, protein-dependent kinase 1; P, phosphorylation site; SMOC2, secreted modular calcium-binding protein 2; ET-1, endothelin 1; NAC, nascent polypeptide-associated complex and coactivator ; -parv, -parvin; HIF1 , hypoxia-inducible factor 1 .

have shown that ILK is required to recruit actin filaments to the plasma membrane at muscle attachment points. In vertebrates, loss-of-function analyses have underscored the importance of ILK in the mediation of protein-protein interactions that are related to cytoskeletal dynamics and in providing a hub for the activation of signaling pathways (Bendig et al., 2006; Knoll et al., 2007; Sakai et al., 2003; Yasunaga et al., 2005). For example, embryonic lethality was observed in *Xenopus laevis* (Yasunaga et al., 2005) and mouse (Sakai et al., 2003) models of *ILK* ablation, and this was attributed to defects in adhesive and migratory mechanics. In the zebrafish embryonic-lethal heart-failure mutant *mainsqueeze*, the binding of ILK to -parvin was disrupted and ILK kinase activity was reduced (Bendig et al., 2006). Similarly, the zebrafish *lost-contact* mutant exhibited poor kinase activity, and cardiac dysfunction (Knoll et al., 2007). Collectively, these findings provide a rich body of evidence that supports the physiological necessity of the adaptor and kinase functions of ILK. However, the lethal nature of *ILK* deletion has made the delineation of its tissue-specific functions challenging. The advent of technologies such as Cre-*lox*-driven recombination and RNA interference has led to experimental strategies that are designed to circumvent this issue. In this section, we elaborate on the specific roles of ILK in several major tissue and/or organ systems, including the musculoskeletal system, smooth muscle, the kidney and liver, the central nervous system, the immune system, and the cardiovascular system (Table 1).

ILK in the musculoskeletal system and the skin

During bone formation, integrin-dependent interactions with the developing bone matrix and the engagement of downstream signaling effectors are both required for the proliferation and differentiation of chondrocytes within the growth plate. Similarly, 1 integrins regulate the adhesion and differentiation of the skin epidermis, have an essential role in the migration and proliferation of cells during hair-follicle morphogenesis, and are centrally involved in skeletal-muscle homeostasis. Thus, the tissues that compose the musculoskeletal system and the skin provide robust environments in which to explore the role of ILK as an effector of integrin-mediated signaling. Several studies have employed Cre-*lox*-driven recombination strategies to functionally delete ILK in chondrocytes (Grashoff et al., 2003; Terpstra et al., 2003), keratinocytes (Lorenz et al., 2007; Nakrieko et al., 2008) and myocytes (Gheyara et al., 2007; Wang et al., 2008; White et al., 2006).

Mice that are devoid of ILK in chondrocytes suffer from dwarfism and chondrodysplasia (Grashoff et al., 2003; Terpstra et al., 2003), which demonstrates the functional requirement for ILK in normal bone growth. Chondrocytes that were derived from animals harboring the chondrocyte-specific ILK deletion suffered from reduced proliferation both in vivo and in culture, as well as from defects in cell adhesion and spreading; however, neither differentiation nor apoptosis were affected. The phosphorylation of Akt and GSK3 was not altered in mutant chondrocytes, which indicates the non-essential nature of the kinase activity of ILK against these two particular substrates under certain circumstances (Troussard et al., 2006). These data do, however, demonstrate a crucial role for ILK-mediated signaling in chondrocyte proliferation and adhesion during normal skeletal growth.

The keratinocyte-specific deletion of *ILK* in mice led to reduced integrin-mediated adhesion and impaired basement-membrane

Table 1. Functions of ILK in normal tissue

Tissue	Observed functions of ILK	Pathological implications of *ILK* knockout	References
Bone (chondrocytes)	Proliferation; adhesion and spreading	Dwarfism	(Grashoff et al., 2003; Terpstra et al., 2003)
Skin (keratinocytes)	Proliferation; adhesion and spreading; migration	Skin blistering; inhibition of hair-follicle development	(Lorenz et al., 2007; Nakrieko et al., 2008)
Skeletal muscle	Adhesion; mechanosensing; myoblast differentiation	Muscular dystrophy	(Gheyara et al., 2007; Huang et al., 2000; Mackinnon et al., 2002; Miller et al., 2003; Postel et al., 2008; Wang et al., 2008; White et al., 2006; Zervas et al., 2001)
Smooth muscle	Contractility; adhesion and cytoskeletal dynamics	Unknown	(Deng et al., 2001; Deng et al., 2002; Erdodi et al., 2003; Huang et al., 2006; Muranyi et al., 2002; Zhang et al., 2007)
Kidney (tubular epithelial cells, podocytes)	EMT; adhesion	Fibrosis; proteinuria	(Dai et al., 2006; El-Aouni et al., 2006; Kanasaki et al., 2008)
Liver (hepatocytes)	Differentiation; survival	Hepatitis	(Gkretsi et al., 2007a; Gkretsi et al., 2007b)
Central nervous system (granule cell precursors, neurons)	Adhesion; migration and polarity; proliferation; survival	Cobblestone lissencephaly	(Belvindrah et al., 2006; Gary et al., 2003; Guo et al., 2006; Guo et al., 2007; Mills et al., 2003; Mills et al., 2006; Naska et al., 2006; Niewmierzycka et al., 2005; Oinuma et al., 2007; Zhou et al., 2004)
Immune system (T cells, macrophages)	Survival; adhesion; migration; proliferation	Unknown	(Chu et al., 2006; Liu et al., 2005; Tan et al., 2002; Troussard et al., 2003)
Cardiovascular system (endothelial cells, cardiomyocytes)	Survival; proliferation; migration and invasion	Dilated cardiomyopathy	(Friedrich et al., 2004; Joshi et al., 2007; Tan et al., 2004; Vouret-Craviari et al., 2004; Knoll et al., 2007; White et al., 2006)

(BM) integrity, and imparted the expression of basal-cell markers on suprabasal cells (Lorenz et al., 2007; Nakrieko et al., 2008). Mutant keratinocytes also exhibited defects in polarity (Lorenz et al., 2007) and proliferation (Nakrieko et al., 2008). Furthermore, in mutant animals, hair-follicle development was severely impaired, which resulted in progressive hair loss (Lorenz et al., 2007; Nakrieko et al., 2008). Cultured ILK-null keratinocytes exhibited defects in attachment, spreading and migration (Lorenz et al., 2007; Nakrieko et al., 2008). One report has suggested that -catenin–Lef1 signaling, Rac1 activation, and the phosphorylation of Akt and GSK3 are not altered in *ILK*-null keratinocytes (Lorenz et al., 2007), whereas another study has reported inhibition of cell proliferation, the abnormal distribution of active Cdc42 to cell protrusions, and reduced Rac1 activation in response to migration induced by the wounding of continuous epithelial cell sheets (Nakrieko et al., 2008). These discordant findings might result from the differential activation of signaling pathways in leading keratinocytes in confluent monolayers compared with those that are plated as single cells (Nakrieko et al., 2008) and serves as a reminder that successfully demonstrating the functional attributes of complex proteins requires careful consideration of relevant model systems. Importantly, ILK has previously been shown to interact with paxillin, parvins and -PIX to modulate Rac1 activation and directional cell movement in various cell types (Filipenko et al., 2005).

Mammalian skeletal muscle exhibits robust ILK expression, particularly at the myotendinous junctions and costameres (Wang et al., 2008). Although initial studies using mice with myocyte-specific ablation of ILK suggested that ILK might be dispensable for the development and homeostasis of skeletal muscle in mammals, these animals displayed severe heart abnormalities that led to early death, which precluded a detailed assessment of skeletal-muscle phenotypes (White et al., 2006). In subsequent investigations of the role of ILK in skeletal muscle, ILK was specifically deleted without affecting cardiac function (Gheyara et al., 2007; Wang et al., 2008). In these studies, the loss of ILK invoked a mild, progressive muscular dystrophy that was characterized by BM detachment, variable myofiber size and fibrosis. Interestingly, ILK-deficient myoblasts showed no defects in myoblast fusion or in actin–muscle-cell-membrane attachment sites, suggesting that ILK functions differently in mammalian myofibers compared with that observed in invertebrates, in which ILK is required for muscle attachment (Mackinnon et al., 2002; Zervas et al., 2001). Exercise exacerbated the muscular pathology, and resulted in architectural disruptions and diminished phosphorylation of insulin-like growth factor 1 receptor (IGF1R) and Akt, which indicates that ILK protects muscle from stress-induced damage via an IGF1R-Akt pathway (Gheyara et al., 2007; Wang et al., 2008).

Similar observations have been reported in non-mammalian vertebrates. The zebrafish mutant *lost-contact*, which lacks a functional *ILK* gene, exhibits a skeletal-muscle phenotype characterized by mechanical instability of skeletal-muscle fibers (Postel et al., 2008). Genetic and morpholino knockdown strategies in this model have demonstrated that ILK is recruited to the myotendinous junctions in a process that requires the presence of laminin in the ECM and 7 integrin in the sarcolemma (Postel et al., 2008). Although ILK is apparently dispensable for the formation of adhesion complexes in the zebrafish, its kinase activity is required for strengthening the muscle-fiber–ECM interaction (Postel et al., 2008). These data indicate that, in contrast to the dispensable nature of ILK kinase activity in invertebrate muscle function (Mackinnon

et al., 2002; Zervas et al., 2001), the kinase activity of ILK is necessary for mechanosensing and signaling in vertebrate skeletal muscle (Postel et al., 2008).

Studies have also identified ILK as an important regulator of myogenic differentiation, although the precise functions of ILK in this context remain controversial. Overexpression of ILK in mouse C2C12 skeletal myoblasts was found to inhibit the expression of myosin heavy chain, and of the transcriptional regulators MyoD and myogenin, leading to impaired formation of multinucleated myotubes (Huang et al., 2000). ILK overexpression also impaired the inactivation of MAP kinase – a necessary step in the initiation of myogenic differentiation – which suggested that ILK might inhibit the differentiation of myoblasts (Huang et al., 2000). Overexpression of ILK in rat L6 myoblasts, by contrast, led to a PI3K-dependent increase in ILK kinase activity and to the stimulation of insulin-induced myogenic differentiation (Miller et al., 2003). The opposing effects of ILK on myogenic differentiation in these two systems might be due to species differences. Alternatively, it has been suggested that the discrepancy results from differential effects on MAPK activation, because MAPK expression and activity were not altered in the L6 myoblasts (Miller et al., 2003).

In summary, the targeted deletion of ILK in chondrocytes, keratinocytes and skeletal muscle demonstrates that there is a close link between ILK and integrin function in these tissues. Furthermore, the studies underscore the importance of ILK signaling in the regulation of cell proliferation, adhesion and motility.

ILK in smooth muscle

The contraction of smooth muscle occurs predominantly through Ca^{2+}/calmodulin-dependent activation of myosin light chain kinase (MLCK) and phosphorylation of myosin light chain (MLC), whereas certain G-protein-coupled receptor agonists elicit contraction in a Ca^{2+}-independent fashion. A series of biochemical studies has implicated ILK in the generation of Ca^{2+}-independent contraction by smooth muscle. Initial investigations identified ILK as a novel MLCK that was isolated from a myofilament fraction of smooth muscle (Deng et al., 2001). ILK was subsequently found to phosphorylate and positively regulate myosin phosphatase target subunit isoform 1 (MYPT1), an inhibitory subunit of myosin light chain phosphatase (MLCP) (Muranyi et al., 2002). Furthermore, two inhibitors of MLCP – protein kinase C (PKC)-dependent phosphatase inhibitor of 17 kDa (CPI-17) and phosphatase holoenzyme inhibitor 1 (PHI-1) – were shown to be phosphorylated and activated by ILK (Deng et al., 2002; Erdodi et al., 2003). Collectively, these studies demonstrate that ILK kinase activity regulates smooth-muscle contraction both directly, by phosphorylating and activating myosin, and indirectly, by inhibiting MLCP via activation of MYPT1 and the MLCP inhibitors PHI-1 and CPI-17.

The mechanism for ILK-mediated contraction of smooth muscle has now been confirmed in cultured cells in response to activation of Gi-coupled receptors. Sustained smooth-muscle contraction by Gi-coupled receptor agonists was shown to involve sequential activation of G i, PI3K and ILK, with direct phosphorylation of MLC by ILK, and CPI-17-mediated inhibition of MLCP (Huang et al., 2006). A role for ILK in stimulus-induced contraction of tracheal smooth muscle has also been reported. The recruitment of ILK, together with PINCH and -parvin, to FAs has been found to regulate actin polymerization that is initiated by the actin nucleating protein N-WASP in response to acetylcholine-stimulated contraction in tracheal smooth muscle (Zhang et al., 2007). These actions dynamically modulate tension development in smooth-muscle tissues and illustrate that, in addition to its kinase function, the role of ILK as an adaptor protein is important for smooth-muscle contractility.

ILK in the kidney and liver

ILK has emerged as an important intracellular mediator of normal organ function in the kidney and liver. In the kidney, the development of tubulo-interstitial fibrosis and glomerular disease has been linked to aberrant expression and regulation of ILK (Blattner and Kretzler, 2005). In a mouse model of unilateral ureteral obstruction (UUO), the overexpression of ILK in renal tubular epithelia was associated with tubular EMT (Li et al., 2003). In the same study, the administration of hepatocyte growth factor to cultured renal tubular epithelial cells inhibited TGF 1-induced ILK expression in vitro and reduced ILK expression in animals with UUO in vivo, which led to the abrogation of the EMT and the attenuation of renal fibrosis (Li et al., 2003).

Podocytes are specialized epithelial cells that have foot processes that interdigitate with one another to form membrane-covered slits (slit diaphragms) surrounding the glomerular capillaries of the kidney. These cells provide structural and adhesive support to the glomerular filtration barrier. The role of ILK in the maintenance of the glomerular filtration barrier has been evaluated in mice that harbor a podocyte-specific deletion of *ILK*. Such mice appear normal at birth, but rapidly develop proteinuria and evidence of pathology (Dai et al., 2006; El-Aouni et al., 2006; Kanasaki et al., 2008). Disease progression was marked by podocyte damage, the loss of slit diaphragm function, glomerulosclerosis and, eventually, renal failure and death (Dai et al., 2006; El-Aouni et al., 2006; Kanasaki et al., 2008). Early, overt cell death of ILK-deficient podocytes was not seen (Dai et al., 2006; El-Aouni et al., 2006), but the localization of 3-integrin, nephrin and -actinin was perturbed, and early foot-process effacement and morphological abnormalities were also observed (Dai et al., 2006; El-Aouni et al., 2006). Furthermore, the formation of ILK–nephrin– -actinin complexes was disrupted in *ILK*-null cells. Interestingly, the podocyte-specific deletion of 1 integrins resulted in a similar, albeit more severe, phenotype (Kanasaki et al., 2008). These studies demonstrate that the functional loss of ILK within a complex system can cause aberrant function that leads to organ failure. This reinforces the need for the site-dependent homeostasis of ILK levels if normal tissue function is to be preserved.

Similar to glomerular cells in the kidney, a tight interaction between hepatocytes and the extracellular matrix that surrounds them is considered to be crucial for normal liver function. The targeted deletion of *ILK* from the mouse liver resulted in the downregulation of differentiation markers (Gkretsi et al., 2007a). Accordingly, *ILK*-null hepatocytes were smaller and less differentiated, and demonstrated a blunted response to the Matrigel-mediated stimulation of differentiation. Such cells showed a decline in cytosolic levels of ILK, PINCH and -parvin during matrix-induced differentiation, whereas levels of FAK and paxillin were elevated, which suggests that ILK and its interactors are dynamically regulated during this process (Gkretsi et al., 2007a). In another study, the adenoviral delivery of Cre recombinase by tail-vein injection to *ILK*-floxed mice resulted in an acute hepatitis that was characterized by inflammation, fatty changes, apoptosis, abnormal mitoses, hydropic degeneration and necrosis (Gkretsi et al., 2007b). Although the elimination of ILK was not organ-specific in this

model, the method of the delivery of Cre recombinase increased the infectivity and *ILK* excision rates in the liver and spleen, in comparison to other organs (Gkretsi et al., 2007b). The deletion of ILK in primary mouse hepatocytes isolated from *ILK*-floxed mice resulted in increased caspase-3 activity and apoptosis, together with decreased expression of PINCH and -parvin (Gkretsi et al., 2007b). Collectively, data derived from hepatocyte-specific deletion studies suggest that ILK has a crucial role in hepatocyte differentiation and survival, and in the maintenance of normal liver function.

ILK in the central nervous system

Defects in integrin function have profound effects on the development of the central nervous system (CNS), which indicates the probable importance of intracellular integrin effectors, such as ILK, in this system. The deletion of *ILK* from the mouse brain using a series of CNS-specific promoters resulted in cortical-lamination defects resembling cobblestone lissencephaly, in neuronal-cell-migration abnormalities, in fragmentation of the basal lamina and in dysregulation of the glial-cell network; often, these mice died prematurely (Belvindrah et al., 2006; Mills et al., 2006; Niewmierzycka et al., 2005). Interestingly, the deletion of ILK did not impact directly on cell survival (Mills et al., 2006; Niewmierzycka et al., 2005), although there was reduced proliferation of granule-cell precursors (GCPs) (Belvindrah et al., 2006; Mills et al., 2006) and the deletion of ILK in primary GCPs inhibited proliferation induced by sonic hedgehog (Shh), a 1-integrin-dependent GCP mitogen that interacts directly with laminin (Blaess et al., 2004; Mills et al., 2006). There was less guanine triphosphate (GTP)-bound Cdc42 in the cerebellum of ILK-deficient mice, and ILK and Cdc42 were required for the 1-integrin-dependent outgrowth of glial processes (Belvindrah et al., 2006). Many of these observations are reminiscent of the defects described in CNS-restricted 1-integrin-knockout mice (Graus-Porta et al., 2001) and provide strong evidence that ILK is a major intracellular mediator of 1-integrin-dependent processes in the brain.

Cellular localization studies have demonstrated that ILK is enriched in neurons, colocalizes with integrins and is preferentially expressed in axonal growth cones (Guo et al., 2007; Mills et al., 2003; Zhou et al., 2004), which suggests that the manipulation of ILK expression may provide mechanistic insights into its role in CNS development and neuronal function. Although studies of CNS-restricted ILK-knockout mice failed to detect differential phosphorylation of Akt or GSK3 compared with wild-type mice (Niewmierzycka et al., 2005), these analyses were carried out using western blots of whole brain extracts. Data indicate that ILK is preferentially expressed in particular regions of the brain (Mills et al., 2006), and detailed immunohistochemical analysis of specific areas of the brain for phosphorylated Akt and GSK3 might reveal ILK-mediated regulation of these substrates.

Using both small-molecule inhibitors and genetic deletion strategies, several reports in cell-culture models have suggested that ILK has a crucial role in neuronal cell functions that depend on the regulation of GSK3 (Guo et al., 2007; Mills et al., 2003; Naska et al., 2006; Zhou et al., 2004). The overexpression of dominant-negative ILK in rat neurons inhibited nerve-growth-factor-stimulated phosphorylation of Akt and GSK3 , thereby reducing Tau-mediated neurite outgrowth (Mills et al., 2003). In hippocampal neurons, GSK3 inactivation and axon formation were mediated by R-Ras and required ILK (Oinuma et al., 2007). The downregulation of ILK expression was found to inhibit axon formation through the elimination or length reduction of the axon; this effect mimics a phosphorylation mutant of GSK3 , and biochemical, and functional studies have positioned ILK upstream of Akt and GSK3 in the development of neuronal polarity (Guo et al., 2007). Genetic deletion of ILK was also found to inhibit depolarization-induced dendrite formation through the regulation of GSK3 phosphorylation (Naska et al., 2006). Finally, ILK-mediated Akt and GSK3 phosphorylation was required for integrin-mediated neuronal survival responses (Gary et al., 2003). Collectively, these data implicate the ILK-Akt-GSK3 signaling axis in the development and function of neurons.

ILK in the immune system

Mononuclear leukocytes exhibit strong ILK expression and the kinase activity of this protein is potently induced by chemokines (Liu et al., 2005), which suggests that ILK is involved in the chemokine-dependent biological activities of leukocytes. T-cell-restricted ILK depletion in mice resulted in an increase in the number of thymic T cells that died, which suggests that the absence of ILK sensitizes these cells to apoptosis (Liu et al., 2005). Enrichment of ILK 'competent' peripheral T cells, a small subset of thymic T cells that escaped Cre-mediated excision of the *ILK* gene and express ILK protein, was also observed, which indicates that *ILK*-null cells suffer a competitive disadvantage in trafficking and/or survival (Liu et al., 2005). Studies in cultured cells have indicated that ILK regulates immune-cell survival via the Akt pathway. For example, ILK-deficient T cells were more sensitive to stress-induced apoptosis and had diminished responses to chemokine stimulation (Liu et al., 2005). Furthermore, chemokine-induced phosphorylation of Akt at Ser473 and Akt activity were suppressed. The ablation of *ILK* in mouse macrophages also led to increased apoptosis and the suppression of Akt phosphorylation and activity, as well as GSK3 phosphorylation (Troussard et al., 2003).

In addition to its importance in leukocyte survival, ILK has been shown to function in the control of leukocyte adhesion, migration and proliferation. Chemokine-stimulated Rac activity was inhibited in ILK-depleted T cells, whereas differential basal levels of Rac activation were not apparent (Liu et al., 2005). Leukocytes depleted of -parvin also showed defects in both adhesion and migration (Yoshimi et al., 2006), although deletion of -parvin in mice did not impair leukocyte migration (Chu et al., 2006). This apparent discrepancy might result from differential signaling in pure populations of cells as compared with complex, mixed cell populations in vivo, in which system redundancies might mask the phenotype. *ILK*-null T cells did not suffer from proliferative deficiencies (Liu et al., 2005), whereas decreased proliferation was observed in ILK-depleted macrophages, which again illustrates the complex cell-type- and context-specificity of ILK function. Collectively, these data support a role for ILK in chemokine-induced survival and motility in leukocytes, and provide strong evidence for ILK kinase function in the regulation of Akt phosphorylation at Ser473 and in the survival in these cells. These studies also suggest that ILK function is required for inflammatory responses and, as such, ILK may be a promising therapeutic target for the regulation of inflammatory disorders (notably, LPS-mediated activation of NF- B and cyclooxygenase 2 (COX2) in macrophages has been shown to require ILK and Akt activity) (Tan et al., 2002).

ILK in the cardiovascular system

Some of the more intriguing findings from targeted ILK deletions in vivo relate to its emerging role in vascular development and cardiac function. The role of ILK in the cardiovascular system has

been reviewed in detail recently (Hannigan et al., 2007), so the intent here is to highlight insights derived from studies using gene-deletion strategies in endothelial cells (ECs) and cardiomyocytes.

Vasculature

EC-restricted ablation of ILK in mice resulted in severe vascular defects that affected the placenta, yolk sac and embryo (Friedrich et al., 2004). The deletion of ILK in ECs in culture subsequently showed that this protein is important for several processes, including cell survival: increased apoptosis and decreased Akt phosphorylation at Ser473 were observed in *ILK*-null mouse ECs (Friedrich et al., 2004). In HUVECs, the T-cadherin-dependent upregulation of Akt Ser473 phosphorylation, as well as -catenin accumulation and cell survival, were shown to be dependent on ILK function (Joshi et al., 2007). Interestingly, the introduction of wild-type ILK, but not constitutively active Akt, rescued the apoptosis and Akt-phosphorylation phenotypes in *ILK*-null mouse ECs, which suggests that, although ILK deletion results in the significant downregulation of Akt phosphorylation at Ser473 in these cells, ILK also functions through Akt-independent pathways to maintain EC survival (Friedrich et al., 2004).

The deletion of ILK also affected cytoskeletal dynamics, proliferation, migration and invasion in ECs. Disorganization of the actin cytoskeleton, disruption of FAs, downregulation of 1 integrins and defects in cell spreading were all observed in *ILK*-null cells both of mouse and bovine origin (Friedrich et al., 2004; Vouret-Craviari et al., 2004). The knockdown of *ILK* by RNAi in HUVECs reduced vascular endothelial growth factor (VEGF)-stimulated proliferation, migration and invasion (Tan et al., 2004), whereas, in *ILK*-null bovine ECs, cyclin-D expression, basal and agonist-stimulated migration, and capillary formation were impaired (Vouret-Craviari et al., 2004). Taken together, the experimental observations in ECs provide further evidence of the role of ILK in cell survival and demonstrate that ILK is involved in agonist-induced cellular functions that are crucial to the maintenance of vascular integrity.

Cardiac muscle

The conditional deletion of *ILK* in mouse cardiomyocytes resulted in the development of dilated cardiomyopathy (DCM) and led to sudden death in the absence of applied stress (White et al., 2006). These *ILK*-null hearts, which were phenotypically similar to those from mice that harbor cardiac-specific deletions of the genes that encode 1-integrin (Shai et al., 2002), melusin (Brancaccio et al., 2003) and focal adhesion kinase (FAK) (Peng et al., 2006), showed a loss of structural integrity, increased interstitial fibrosis and reduced expression of 1 integrin (White et al., 2006). The downregulation of phosphorylation of FAK at Tyr398, a binding site for cell-adhesion effectors, was also observed. Akt phosphorylation at Ser473 was also markedly diminished in these animals. Together, these data illustrate the multiple roles of ILK in the maintenance of cell survival and function in the cardiovascular system. Indeed, point mutations in laminin and ILK have recently been identified that directly cause dilated cardiomyopathy DCM in humans through simultaneous defects in ECs and cardiomyocytes (Knoll et al., 2007); these provide a genetic link between aberrant ILK function and cardiovascular disease.

The main message from the studies described thus far in this article is that ILK is a multifunctional protein that can regulate several key cellular processes. Although no single tissue provides insight into all the functions of ILK, gene-deletion strategies have

Table 2. ILK expression in human malignancies

Malignancy	Comments	Positivity* (%)	Reference
Melanoma	Strong expression of ILK was associated with tissue thickness and was inversely correlated with 5-year survival	95.5	(Dai et al., 2003)
Colon cancer	ILK expression levels correlated with tumor invasion, grade and stage; higher levels in metastatic tumors, no staining in normal epithelium	97.5	(Bravou et al., 2003)
Colon cancer	Overexpression of ILK in malignant acini compared with normal crypts		(Marotta et al., 2003)
Colon cancer	ILK expression levels correlated with tumor invasion, grade and stage; higher levels in metastatic tumors	98.4	(Bravou et al., 2006)
Colorectal cancer	ILK expression in the majority of tumors	80	(Huang et al., 2007)
Gastric cancer	Strong expression in the majority of primary tumors that were associated with tumor cell invasion and nodal metastasis; no expression in non-neoplastic gastric epithelia	69	(Ito et al., 2003)
Non-small-cell lung cancer	ILK expression significantly associated with tumor grade and stage, and lower 5-year survival; ILK expression indicates a poor prognosis	96	(Takanami, 2005)
Non-small-cell lung cancer	Strong cytoplasmic staining in a subset of tumors that is associated with poor prognosis	24.5	(Okamura et al., 2007)
Anaplastic thyroid cancer	ILK highly expressed in tumor tissue, but not in normal thyroid tissue	81	(Younes et al., 2005)
Squamous cell carcinoma of head and neck	Tumors stained for ILK; normal tongue tissue was negative for ILK; ILK is overexpressed in tumors	88.2	(Younes et al., 2007)
Pancreatic cancer	Strong ILK expression was a significant (negative) prognostic indicator	65	(Sawai et al., 2006)
Ovarian cancer	ILK expression increased with tumor progression; normal epithelium was negative for ILK	100	(Ahmed et al., 2003)
Prostate cancer	ILK expression increased with tumor progression; inversely correlated with 5-year survival	44	(Graff et al., 2001)
Mesothelioma	ILK expression at varying levels in tumor samples; normal mesothelial cells and lung parenchyma were negative	87.9	(Watzka et al., 2008)
Ewing sarcoma	Expression observed in all samples	100	(Chung et al., 1998)
Primitive neuroectodermal tumor	Expression observed in all samples	100	(Chung et al., 1998)
Medulloblastoma	Expression observed in all samples	100	(Chung et al., 1998)

*The number of samples that stained for ILK, expressed as a percentage.

shown that individual tissues collectively illustrate the importance of ILK in several capacities. Thus, ILK functions in bone, skin and the CNS to regulate processes that are related to cell adhesion, migration and proliferation. By contrast, cells of the immune and cardiovascular systems rely on the adaptor and kinase capacities of ILK to maintain cell survival and tissue function.

The role of ILK in cancer biology

Significant defects in normal development and tissue homeostasis occur when ILK is deleted; by contrast, the overexpression or constitutive activation of ILK either in cell culture or in transgenic mouse models results in oncogenic progression (Dillon et al., 2007; Hannigan et al., 2005; Legate et al., 2006). The expression of ILK is often elevated in human malignancies, and correlates with tumor stage and grade (Table 2). Importantly, strong ILK expression predicts poor patient survival in several types of cancers (Table 2) (Dai et al., 2003; Graff et al., 2001; Okamura et al., 2007; Takanami, 2005; Yau et al., 2005). The cause of elevated ILK expression is not clear and might vary among tumors; accumulating evidence, however, suggests that the oncogenic capacity of ILK derives from its regulation of several downstream targets that promote cell proliferation, survival and migration (Fig. 2). It is perhaps not surprising that many attributes of the cell-signaling functionality of ILK have been delineated using various models of malignancy. Inhibiting the overexpression of ILK and returning its expression and/or activity to normal levels might, therefore, be of substantial therapeutic benefit to cancer patients. In this section, we describe two approaches to downregulate ILK expression in cancer cells: genetic inhibition of ILK expression by treatment with siRNA or antisense oligonucleotides, and pharmacologic inhibition by treatment with small-molecule inhibitors.

Genetic inhibition of ILK function in cancer cells

The emergence of technologies that inhibit gene expression in a targeted fashion, including RNA interference and antisense oligonucleotides, has yielded powerful tools for examining the mechanistic and functional consequences of ILK depletion on oncogenic growth and progression. Importantly, siRNA-mediated depletion of ILK impairs the phosphorylation of Akt at Ser473 in several cancer-cell models (Basaki et al., 2007; Duxbury et al., 2005; Edwards et al., 2006; Liu et al., 2006; McDonald et al., 2008; Tan et al., 2004), which results in the induction of apoptosis (Duxbury et al., 2005; McDonald et al., 2008; Shi et al., 2007). In addition, knockdown of ILK impaired the migration and invasion of cancer cells (Liu et al., 2006; Shi et al., 2007; Wong et al., 2007) under standard growth conditions and subsequent to agonist stimulation (Lin et al., 2007; Shi et al., 2007). Reduced stress-fiber formation and defects in adhesion and spreading were seen in ILK-depleted melanoma cells, and this might underlie some of the observed migration and invasion defects (Wong et al., 2007). The stable knockdown of ILK in melanoma cells also impaired the growth of ILK-depleted xenografts (Wong et al., 2007), which demonstrates the requirement of ILK for tumor progression in vivo. Collectively, these studies illustrate the dependence of cancer cells on the kinase and adaptor functions of ILK for oncogenic processes such as survival and invasion.

Antisense oligonucleotides that are complementary to *ILK* have provided another genetic strategy for the investigation of the functional consequences and potential therapeutic benefit of downregulating aberrant *ILK* expression during oncogenic progression. Similar to siRNA-mediated downregulation of *ILK*, *ILK* antisense-oligonucleotide (*ILK*-AS) treatment of glioblastoma cells reduced *ILK* expression and the phosphorylation of Akt at Ser473 (Edwards et al., 2005; Edwards et al., 2006), and induced apoptosis (Edwards et al., 2005). Furthermore, *ILK*-AS treatment of mice that have established glioblastoma xenografts resulted in stable disease, whereas the tumor volume in control animals escalated (Edwards et al., 2005). These data suggest that ILK imparts a kinase-mediated cell-survival function on glioblastoma cells and indicate that *ILK*-AS might be an effective agent for blunting ILK-mediated tumor progression.

The therapeutic efficacy of inhibiting *ILK* expression has raised the possibility of treating cancers by targeting *ILK* expression in combination with other therapeutic modalities. Cell-based studies have been conducted that use *ILK*-AS in combination with inhibitors of Raf1 and MEK, thus providing tandem regulation of survival and proliferative signaling pathways (Edwards et al., 2006). These investigations showed that *ILK*-AS and the Ras-MAPK inhibitors had a synergistic effect on glioblastoma-cell viability as measured using the Chou and Tabaley median-effect method (Edwards et al., 2006). Similar results were obtained with *ILK* siRNA in combination with a MEK inhibitor (Edwards et al., 2006). Likewise, the downregulation of *ILK* expression using RNAi in pancreatic adenocarcinoma cells resistant to gemcitabine, a nucleoside analog used as a chemotherapeutic agent for the treatment of pancreatic cancer, induced caspase-3-mediated apoptosis and chemosensitization to gemcitabine (Duxbury et al., 2005). Interestingly, the overexpression of constitutively active myristoylated Akt was sufficient to induce significant recovery of gemcitabine resistance after ILK depletion (Duxbury et al., 2005). These studies point to a pro-survival function for ILK in these malignancies and reinforce the concept that therapeutic agents that target ILK might be most efficacious when used together with conventional chemotherapy or other targeted agents.

Mouse models in which ILK is knocked out in a tissue-specific manner also demonstrate that ILK is required for oncogenic progression. For instance, Neu-oncogene-induced mammary-tumor formation is dramatically delayed in the *ILK*-null genetic background (Bill Muller, McGill University, Quebec, Canada, personal communication). Similarly, carcinogen-induced and colitis-associated intestinal-tumor formation is markedly diminished in the absence of ILK (Assi et al., 2008). The mechanism of action of ILK in cancer cells remains to be fully elucidated; however, substantial downregulation of phosphorylation of Akt at Ser473 has been observed in the mammary-tumor model and diminished cyclin-D expression has been reported in the intestinal model, which suggests that ILK-mediated cell survival is at least a part of the story.

Pharmacological inhibition of ILK function

The inhibition of ILK expression using siRNA and *ILK*-AS has provided valuable insights into the role of ILK in tumor progression, but, because these technologies necessarily downregulate the expression of a multifunctional protein, they can give rise to unanticipated consequences. Moreover, the use of these technologies in the clinical setting remains technically challenging. The development of potent, specific small-molecule inhibitors of ILK has provided an essential tool with which to validate the biological functions of ILK and test the therapeutic efficacy of inhibiting ILK activity without inadvertently affecting its non-kinase functions. The inhibitors of ILK are ATP analogs that compete with endogenous

ATP for binding at the active site of the ILK kinase domain. The generic structure for the KP15792 class of inhibitors has been published (Troussard et al., 2006) and two derivatives, the first-generation compound KP-392 and its more potent successor, QLT-0267, have been widely used (Jin et al., 2008; Koul et al., 2005; Liu et al., 2006; Mills et al., 2003; Troussard et al., 2006; Younes et al., 2005; Younes et al., 2007). A third inhibitor of ILK, QLT-0254, is a derivative of QLT-0267 (Yau et al., 2005). Although the ILK inhibitors have been shown to be potent and selective (Koul et al., 2005; Troussard et al., 2006; Younes et al., 2005), these compounds, similar to most small-molecule kinase inhibitors (e.g. imatinib), interact with a highly conserved target and there is growing appreciation that such inhibitory molecules cannot be absolutely specific (Bantscheff et al., 2007). Nevertheless, kinase inhibitors remain invaluable both as experimental tools and as effective therapeutic modalities in the clinic.

The consequences of pharmacological inhibition of ILK kinase activity have now been investigated in several tumor models and common themes have emerged. Importantly, inhibition of Akt phosphorylation at Ser473 has been shown in a number of human cancer-cell models (Cruet-Hennequart et al., 2003; Edwards et al., 2008; Koul et al., 2005; Liu et al., 2006; Persad et al., 2000; Rosano et al., 2006; Tan et al., 2004; Troussard et al., 2006; Younes et al., 2005; Younes et al., 2007). Interestingly, the activity of ILK from normal (non-tumorigenic or untransformed) epithelial and mesenchymal cells was sensitive to the inhibitor, but Akt phosphorylation at Ser473 and apoptosis were unaffected. These findings suggest that normal cells are able to circumvent ILK inhibition, whereas tumor cells become dependent on ILK (Troussard et al., 2006).

The downstream consequences of pharmacological inhibition of ILK kinase activity have also been evaluated in several tumor types. Treatment with ILK inhibitors has resulted in reduced proliferation (Edwards et al., 2008; Koul et al., 2005; Younes et al., 2005; Younes et al., 2007), the induction of apoptosis (Edwards et al., 2008; Troussard et al., 2006; Younes et al., 2005; Younes et al., 2007), the inhibition of motility or invasion (Koul et al., 2005; Rosano et al., 2006; Rosano et al., 2005; Troussard et al., 2000) and a reduction in angiogenesis (Edwards et al., 2006; Koul et al., 2005; Tan et al., 2004). The inhibition of ILK kinase activity also blocked nuclear translocation and Wnt-induced -catenin stabilization in tumorigenic cells (Oloumi et al., 2006). Indeed, ILK inhibitors represent the first small-molecule inhibitors of Wnt-induced -catenin activation (Oloumi et al., 2006). Thus, as in normal tissue, the precise mechanisms by which ILK perpetuates malignant progression might be dependent on cell and tissue context, as well as on specific cues that are present in the surrounding micro-environment.

The biological activity of ILK inhibitors in cells in culture has stimulated interest in the potential therapeutic benefits of inhibiting ILK kinase activity in vivo. Of crucial importance for future clinical application, the ILK inhibitors are tolerated well and exhibit no apparent toxicity (Edwards et al., 2008; Tan et al., 2004; Yau et al., 2005; Younes et al., 2005). Suppression of tumor growth and angiogenesis following treatment with ILK inhibitors has been demonstrated in several xenograft models (Edwards et al., 2008; Tan et al., 2004; Yau et al., 2005; Younes et al., 2005). The acute administration of an ILK inhibitor in an orthotopic pancreatic model rapidly decreased phosphorylation of Akt at Ser473, as well as the phosphorylation of downstream targets, and showed greater efficacy with gemcitabine (Yau et al., 2005). ILK-inhibitor treatment of metastatic orthotopic lung-cancer xenografts in mice specifically decreased the level of Akt phosphorylation at Ser473 and increased tumor necrosis (Liu et al., 2006). Furthermore, a combination of ILK inhibition and treatment with cisplatin increased survival significantly more than either treatment alone. The inhibitor-mediated suppression of Akt Ser473 phosphorylation and induction of apoptosis in both tumor cells and tumor-associated endothelial cells were also observed in a thyroid xenograft model (Younes et al., 2005). The administration of a single dose of ILK inhibitor in combination with gemcitabine resulted in a significant increase in acute apoptosis (Younes et al., 2005), which suggests that ILK inhibitors might be efficacious in combination with conventional chemotherapeutic agents in patients with pancreatic cancer. Most recently, the analysis of EGFR-resistant mesenchymal hepatoma-cell lines in vitro and in an in vivo xenograft model revealed that transfection with kinase-inactive ILK increased the sensitivity of cells to the EGFR inhibitors erlotinib, gefitinib and cetuximab, which demonstrates that inhibition of ILK activity might represent a novel mechanism for overcoming resistance to EGFR inhibitors (Fuchs et al., 2008). These studies show that specific inhibitors of ILK kinase activity result in growth arrest and apoptosis in cells in culture and in vivo, and provide support for ILK as a therapeutic target in human cancer.

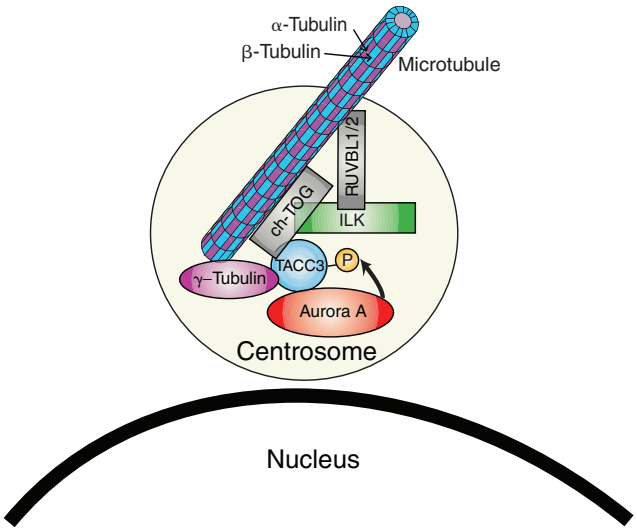

Fig. 3. ILK also localizes to the centrosome. Data from a SILAC-based unbiased proteomic screen place ILK in the centrosome, and indicate that it interacts with tubulin and tubulin-binding proteins to aid in the organization of the mitotic spindle. Binding partners of ILK include -tubulin, -tubulin, RUVBL1 and RUVBL2. The inhibition of ILK activity or expression inhibits Aurora-A–TACC3–ch-TOG interactions, which indicates that ILK might be important in mitotic-spindle assembly.

New directions for ILK – a role in mitosis

Our understanding of the role of ILK in the regulation of multiple signaling pathways has advanced considerably in recent years; however, the precise mechanisms by which ILK affects cellular processes remain to be fully characterized. Indeed, recent data that demonstrate the reliance of tumor cells, but not their non-tumorigenic or untransformed counterparts, on ILK for full activation of Akt (Troussard et al., 2006) suggest that the regulation of ILK-dependent signaling pathways in different cell types and cellular contexts is inherently complex. It has become increasingly clear, however, that a network of protein-protein

interactions forms the cornerstone for ILK-mediated control of cell spreading, migration, growth, cell-cycle progression and survival. We recently undertook a global analysis of ILK-protein interactions by mapping the ILK 'interactome' by using a SILAC (stable isotope labeling with amino acids in cell culture)-based proteomic strategy (Dobreva et al., 2008). Our unbiased proteomic screen has yielded several novel interactions of ILK, including an interaction with Rictor (McDonald et al., 2008), which is a regulator of cytoskeletal dynamics (Sarbassov et al., 2004) and a defining member of mTORC2 (a complex that is implicated in the phosphorylation of Akt; see above for further details) (Sarbassov et al., 2005).

In addition, our analysis revealed that several centrosomal and mitotic-spindle proteins are binding partners of ILK; these included ch-TOG, -tubulin, -tubulin and RUVBL1 (Fig. 3) (Dobreva et al., 2008). These unexpected interactions indicate that ILK might function as part of a complex in centrosomes. This is interesting because several reports have suggested that ILK has a role in mitosis. For example, it has been shown that inhibition of ILK causes a G2-M-phase arrest in glioblastoma-cell lines (Edwards et al., 2008; Koul et al., 2005), that ILK regulates mitotic cell death after irradiation (Monferran et al., 2008), and that siRNA against *ilk* in *Drosophila* causes abnormal mitotic spindles, chromosome abnormalities and lagging chromatids (Bettencourt-Dias et al., 2004).

More detailed analysis demonstrated that ILK could be found in FAs and centrosomes simultaneously in interphase cells (Fielding et al., 2008). Moreover, ILK colocalized with ch-TOG and RUVBL1 in centrosomes at all phases of mitosis, whereas -parvin was absent from the centrosome, suggesting that ILK forms distinct complexes in different subcellular compartments. The inhibition of ILK activity or expression induced dramatic defects in mitotic-spindle organization and inhibited Aurora-A–TACC3–ch-TOG interactions, which indicates that ILK might be important in mitotic-spindle assembly (Fielding et al., 2008). The in vivo mitotic function of ILK has not yet been thoroughly investigated, although removal of ILK from hepatocytes in vivo has been shown to cause mitotic defects (Gkretsi et al., 2007b) and several studies have reported proliferative defects upon ILK depletion (Grashoff et al., 2003; Mills et al., 2006; Nakrieko et al., 2008; Terpstra et al., 2003). As with other functions of ILK, it is likely that the role of ILK in mitosis will exhibit cell and/or tissue specificity, but is likely to have an important role in cancer-cell mitosis.

Conclusion and perspectives

Since its discovery more than a decade ago, ILK has been extensively studied and is characterized as a complex, multifunctional protein that dynamically regulates signals derived from integrin-matrix interactions or growth-factor-receptor stimulation through a combination of intermolecular interactions and kinase activity. The evaluation of ILK function across several organ systems using tissue-specific deletion strategies has demonstrated important contributions of ILK to many cellular processes, including survival, proliferation, differentiation, adhesion, migration and contractility. The precise roles of ILK in maintaining homeostasis of a particular tissue are dependent on contextual cues. Significant defects in normal development and tissue homeostasis occur when the gene that encodes ILK is deleted, whereas the expression of ILK is often elevated in human malignancies. ILK has important roles in regulating an array of biological processes that are crucial to the progression of cancer, and agents designed to interfere with aberrant ILK expression and activity are being evaluated.

Future studies of ILK will need to examine its function in the context of the many direct and indirect interactions present in the ILK 'interactome'. A better understanding of which interactions are important for normal tissue function relative to those interactions that are crucial for the progression of human malignancies will be required if ILK is to be effectively targeted (e.g. the ILK-Rictor interaction). Finally, it will be of great interest to delineate the exact functions and targets of ILK in centrosomes during the mitotic process. It remains to be seen whether documented signaling targets of ILK, especially Akt, GSK3 and catenin, are targeted by centrosomal ILK. Our data suggest independent roles of ILK in FAs and centrosomes, and it will be interesting to determine whether centrosomal ILK functions are altered in cells in which integrin function has been compromised, particularly as mutations in the cytoplasmic tail of 1 integrin result in mitotic-spindle defects (Reverte et al., 2006). Resolving these issues is important because mitotic defects are hallmarks of malignant cells.

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