

Review

Integrin Linked Kinase (ILK) and its Role in Liver Pathobiology

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Integrin linked kinase (ILK) is a vital signaling protein ubiquitously expressed throughout the body. It binds to intracellular integrins to help promote signaling related to cell adhesion, apoptosis, proliferation, migration, and a plethora of other common cellular functions. In this review, ILK's role in the liver is detailed. Studies have shown ILK to be a major participant in hepatic ECM organization, liver regeneration, insulin resistance, and hepatocellular carcinoma.

Key words: Partial hepatectomy; Hepatocyte proliferation; Termination of liver regeneration; Hepatocellular carcinoma; Glucose/carbohydrate metabolism

INTRODUCTION

Integrin Linked Kinase and Integrins

The integrins encompass a large family of adhesion molecules that regulate a myriad of intracellular signaling pathways related to cell migration, survival, proliferation, and differentiation^{1,2}. Integrins are vital proteins as they are responsible for communicating between the intracellular actin cytoskeleton and the extracellular matrix (ECM). These transmembrane heterodimer proteins are made up of alpha and beta chain subunits with the extracellular tail of the alpha chain binding the cell to the ECM, and the cytoplasmic tail on the beta chain interacting with various adaptor and signaling proteins¹.

Integrin linked kinase (ILK) is a crucial signaling protein that interacts with the cytoplasmic domains of the $\beta 1$ and $\beta 3$ integrin chains. ILK, a PI3-kinase-dependent protein, is considered an adaptor that propagates signal transduction from the extracellular adhesion sites to the intracellular signaling targets³. Along with PINCH, and Parvin, ILK forms a heterotrimeric focal adhesion (FA) complex that passes signals down through the cell from the integrins (Fig. 1). ILK has been shown to profoundly affect cell morphology, proliferation, migration, adhesion, and assembly of ECM proteins⁴⁻⁷.

Discovery of ILK and the Debate Over its Kinase Activity

ILK was initially discovered in 1996 by Hannigan et al.⁴, where the authors proposed that ILK is a receptor-proximal protein kinase regulating integrin-mediated signal transduction. Initial studies showed ILK expression was vital during embryonic development and tissue homeostasis as it was found to be involved in signaling pathways involving cell adhesion, apoptosis, proliferation, migration, and a plethora of other common cell functions⁸⁻¹⁰.

Based on the fact that ILK has an amino acid sequence suggestive of kinase functionality, for the next 10 years, debate in the scientific community occurred regarding whether ILK was a bona fide kinase, as ILK was thought to phosphorylate Akt¹¹. However, following extensive studies, it was revealed that ILK lacks several important conserved kinase motifs, specifically the amino acid residues essential for phosphotransferase activity¹²⁻¹⁴. Hence, it is now accepted that when integrins signal through ILK, Akt phosphorylation occurs indirectly through regulation of the protein kinase mechanistic target of rapamycin (mTOR), and not directly through ILK^{15,16}. Additional studies have not been able to prove that ILK can phosphorylate any other substrate, and consequently, ILK is

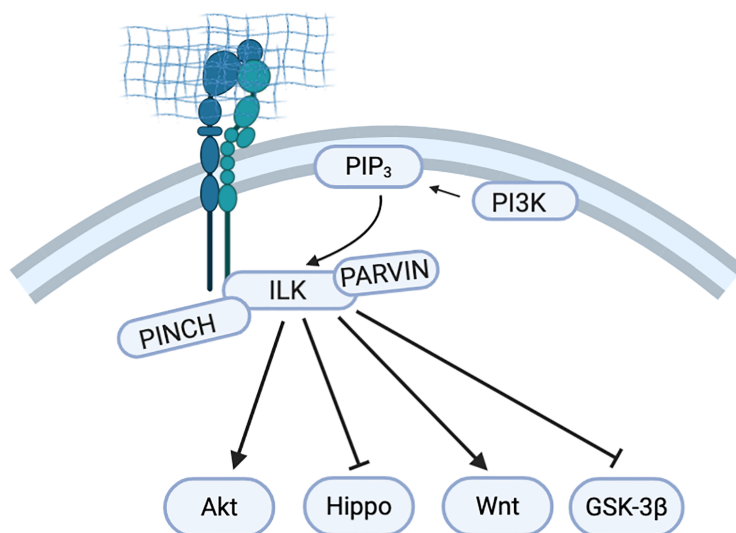


Figure 1. Integrin/integrin linked kinase (ILK) signaling pathway showing localization of ILK in the focal adhesion (FA) complex, and vital downstream targets known to affect cell morphology, proliferation, migration, adhesion, and assembly of extracellular matrix (ECM) proteins.

considered to be a pseudokinase¹³. Currently, there are thought to be about 60 different pseudokinases in existence. Pseudokinases, such as ILK, generally lack one or more amino acids required to phosphorylate protein substrates, rendering them structurally similar but functionally inactive¹⁷.

Functions of ILK

Previous studies have revealed that ILK's main function as a ubiquitously expressed protein is to organize the actin cytoskeleton during development. This has been observed in both *Caenorhabditis elegans* and *Drosophila*, where deletion of the ILK ortholog, PAT-4, causes muscle detachment and early lethality^{18,19}. Similar results were observed in mice, where deletion of ILK causes failure to organize the actin cytoskeleton, resulting in embryonic lethality⁷.

The most commonly observed cellular localization of ILK is below the cellular membrane, where integrin adhesion sites occur. However, ILK has also been observed to be present in the nucleus; COS-1, MCF-7, HeLa cells, and keratinocytes all reportedly contain nuclear ILK^{20–22}. Despite various studies showing nuclear ILK expression, the nuclear aspect of its function is not well understood. Acconcia et al. hypothesized that ILK is important for nuclear integrity as ILK disruption led to altered morphology and abnormal lamin A/C distribution and, further, that ILK can associate with chromatin and act as a suppressor for the *CNKS3* gene in MCF-7 cells²¹. The authors also described a nuclear localization sequence that, when mutated, inhibits ILK's ability to translocate to the nucleus.

Within the context of the liver, ILK has been observed to play various roles in phenomena including fibrosis^{23,24}, regeneration^{23,25–27}, insulin resistance^{28–30}, and cancer^{31–33}.

INTEGRIN LINKED KINASE AND THE LIVER

ILK and the Hepatic ECM

The ECM in the liver plays a major role in the overall microenvironment for hepatocytes as well as other hepatic cells. ECM components are responsible for dictating the stiffness of the matrix³⁴ and can have an effect on the homeostasis within the liver through basic functions of proliferation, migration, differentiation, and cell–cell or cell–matrix adhesion³⁵.

In the presence of persistent damage, whether that be through exposure to a virus or chemical/toxic agents such as drugs or alcohol, hepatic ECM responds with continuous remodeling of the matrix and excessive accumulation of matrix proteins, such as collagens, fibronectins, and laminins, as well as proteoglycans and carbohydrates³⁶. Because of the critical role ILK plays in intracellular signal transduction from the ECM, studies have been conducted to elucidate the possible involvement of ILK in the regulation of the hepatic matrix in the context of injury.

In a study that observed stellate cell activation in fibrogenesis, it was detailed that ILK overexpression in stellate cells mediates Rho-GTPase-dependent effects on collagen and smooth muscle alpha actin expression²⁴. The expression of ILK was greatly increased between quiescent and activated stellate cells, suggesting that the ECM–cell crosstalk could be highly dependent on ILK to regulate matrix changes.

In fact, the potential important relationship between the ECM and ILK within the liver prompted us to study the effects of specifically deleting ILK from hepatocytes (hep-ILK-KO) in mice. Within the context of hepatocytes, the ECM is an important determinant of differentiation and proliferation. In our previous research, we showed that after deletion of ILK by use of the LoxP/Cre system with Cre recombinase under the control of the alpha fetoprotein enhancer/albumin promoter, mice were born normal but soon developed histological abnormalities²³. These abnormalities included a massive deposition of ECM surrounding each hepatocyte, as evidenced by a simple reticulin stain. However, there was no formation of nodules or any other histological evidence of cirrhosis. Interestingly, liver weight-to-body ratio was increased by approximately 50%.

In order to further understand how the ECM of the liver was responding to deletion of ILK in the hepatocytes, we have now investigated the gene expression patterns for the general ECM (all proteins except collagen) and the various collagen proteins in hep-ILK-KO and wild-type (WT) mice (Fig. 2). Mice were generated, and microarray analysis was performed as previously described²³.

In hep-ILK-KO mice, there was decreased mRNA expression of syndecans 1, 2, and 4. A decrease in syndecans, proteins involved in cell–cell and cell–matrix adhesion, can point to ILK playing a vital role in the communication between hepatocytes and cells associated with synthesis of components of the hepatic ECM. However, there was also an increase in the RNAs encoding perlecan, also involved in cell–ECM adhesion, as well as a massive increase in the presence of various collagen RNAs, especially those of Col3a1, Col4a1, and Col1a2. These collagens are synthesized exclusively by stellate cells, correlate well with the increased matrix deposition of collagen we previously observed throughout the parenchyma of the liver in the hep-ILK-KO mice, and demonstrate that ILK provides a crucial signaling link from hepatocytes to stellate cells, regulating the quantity and kind of ECM proteins produced by stellate cells in order to maintain their quiescence in normal liver. In the absence of such signaling, stellate cells appear to become activated and enhance their production of collagens, a finding that has major implications for the pathogenesis of liver cirrhosis as cirrhosis/fibrosis only occurs in conditions associated with persistent loss of hepatocytes. Hence, it is reasonable to hypothesize that chronic loss

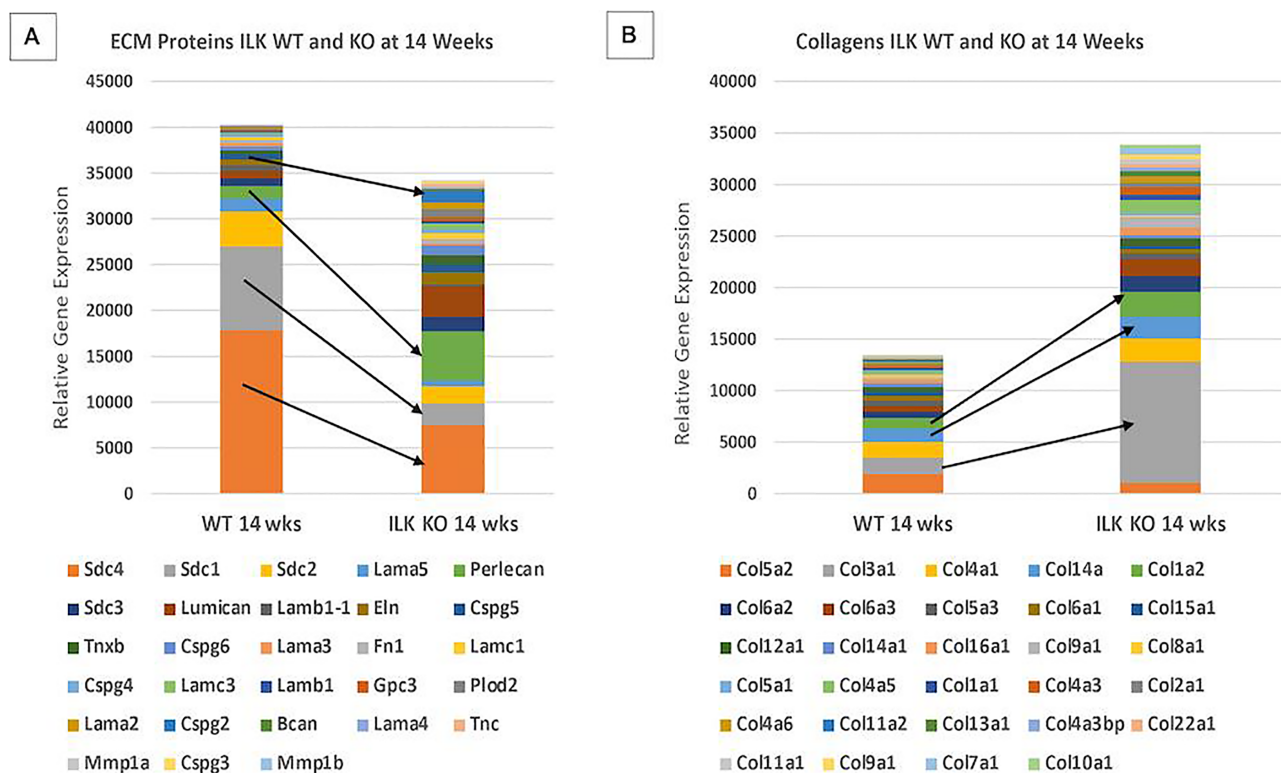


Figure 2. Differences in expression of mRNAs between hep-ILK knockout (KO) and wild-type (WT) mice at 14 weeks of age for (A) general ECM proteins (except collagens) and (B) collagens. Each protein is depicted in a different color. Size represents the absolute value from the array.

of hepatocytes in disease states leads to the generation of “orphan” stellate cells that have minimal contact with hepatocytes. We suggest these “orphan” stellate cells could behave similarly to the stellate cells in the hep-ILK-KOs (i.e., “orphan” stellate cells in disease states), devoid of any regulation by hepatocyte ILK, become “uninhibited,” and continue to produce excess collagen proteins, as seen in the hep-ILK-KO mice.

ILK and Liver Regeneration

The hepatic matrix is a main regulator of cellular proliferation in the liver. Upon partial hepatectomy (PHx), an increase of hepatocyte growth factor (HGF), activation of the MET (HGF) receptor, and concomitant activation of epidermal growth factor receptor (EGFR) occur within 30 min after PHx and induce hepatocyte proliferation. Proliferation of hepatocytes and other nonparenchymal cells ceases within 6–8 days³⁷, an event that is highly dependent on the communication between the ECM, hepatocytes, and hepatic stellate cells^{37–39}.

In normal mice, after PHx, these regenerative activities cease when liver has grown back exactly to the original mass and without exceeding it. However, in hep-ILK-KO mice, following PHx, we observed an enhanced cell proliferation of both hepatocytes and cholangiocytes, as well as hepatomegaly, with the final liver size at the end of regeneration (14 days) exceeding the original prehepatectomy liver mass^{23,25}. Our data show that at 14 days post-PHx, hep-ILK-KO livers grew back to 158% of the original weight, indicating that there was an altered process for termination of regeneration. This enhanced hepatocyte proliferation in the hep-ILK-KO mice was supported by changes in various cell cycle genes. There was an increased expression of c-Myc and decreased levels of CDK2. Additionally, we saw activation of the hippo pathway, with an increase in phosphorylated YAP, which has been associated with higher cellular proliferation²⁵.

Similar results were observed in hep-ILK-KO mice that were subjected to phenobarbital (PB) administration²⁷. Over a 10-day course of PB administration, there was a threefold increase in liver-to-body weight ratios compared to control mice as well as a significant increase in the number of mitotic cells.

The role of ILK in ECM signaling via acetaminophen (APAP) toxicity and compensatory regeneration was also investigated⁴⁰. Using the hep-ILK-KO mice, it was observed that there was attenuated injury after 6 and 24 h of APAP overdose in the KO mice compared to control. By histological examination, there was lower centrilobular necrosis. Measurement of alanine aminotransferase (ALT) corroborated these data showing extensive damage in WT mice compared to hep-ILK-KO. Interestingly, there was improved liver regeneration after APAP-

induced injury by Ki-67 staining and Western blot analysis of PCNA, cyclin D1, CDK4, and phosphorylated Rb⁴⁰. These data strongly correlate with the previous study that shows ILK removal promotes the proliferation of hepatocytes and regeneration of the liver after PHx.

The results of removal of ILK in hepatocytes clearly indicate the essential role that ILK plays in successfully transmitting signals between cells and the hepatic ECM.

ILK and Hepatic Insulin Resistance

Inhibition of hepatic gluconeogenesis mediates insulin-stimulated clearance of blood glucose and is, overall, a major contributor to glycemic regulation⁴¹. With worldwide obesity rates tripling in the past 50 years, studies surrounding insulin resistance and type 2 diabetes, both considered consequences of obesity, have been extensively studied. It has been shown that with consumption of a high-fat diet (HFD), hepatic insulin resistance occurs, which then disrupts the process of gluconeogenesis and overall clearance of blood glucose⁴².

As discussed, the hepatic ECM is a regulator of various liver functions that maintain homeostasis, including whole-body glucose levels. On an HFD, there is increased hepatic triglyceride synthesis and storage and, therefore, an increase in the accumulation of hepatic lipids^{43,44}. This results in the expansion of the hepatic ECM²⁸ and liver damage associated with increases in ECM proteins⁴⁵.

In fact, Williams et al. detailed a study in which hepatocyte-specific deletion of ILK (hep-ILK-KO, ILK^{lox/lox} Albcre) in mice on an HFD led to insulin sensitization²⁸. Results showed that hepatic insulin action improved in HFD hep-ILK-KO mice as shown by a 50% increase in the glucose infusion rate, as well as reductions in liver lipid and triglyceride accumulation²⁸. Additionally, the gluconeogenic genes, G6pc and Pepck, were observed to be increased at basal levels in the HFD hep-ILK-KO mice compared to controls and were then greatly suppressed with an insulin stimulus.

Multiple studies have shown that global transgenic downregulation or deletion of ILK in mouse models showed initiation of insulin resistance^{29,46}. In the global ILK downregulation model, the authors observed an inversely correlated increase in hepatic gluconeogenesis in accordance with other studies²⁸. Hepatic expression of ILK in the control mice was also observed to decrease while on the HFD^{28,29}, suggesting that expression is downregulated as a consequence of overnutrition and is directly related to hepatic insulin action²⁸. Another study demonstrated that GSK3 β inhibition improved hepatic insulin resistance, which correlates well with the previous studies as ILK normally promotes inhibition of GSK3 β ^{47,48}.

Trefts et al.³⁰ detailed the metabolic and glucoregulatory role of ILK and showed that hepatocyte ILK is required for glucose homeostasis through cellular signaling events

relating to ECM functions. RNAseq data in hep-ILK-KO mice revealed an increase in genes involved with integrin functions, FAs, and actin cytoskeleton regulation. There was also a decrease in RNA of proteins associated with mitochondrial function, which resulted in decreased ATP levels, and therefore stimulation of glycolysis, as well as an increase in AMP-activated protein kinase activation. Overall, the results support the hypothesis that disruption of hepatic glucose homeostasis can result from the inability of ECM signals to be transmitted through ILK and integrin receptors³⁰.

ILK and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common types of cancer, resulting in over 750,000 deaths in the US in 2018, with the incidence tripling over the last four decades^{49,50}. Patients with underlying liver diseases such as fibrosis or hepatitis B or C infection are at an increased risk of developing HCC, with the main treatment option being liver transplantation. In parallel with the increase in obesity and development of type 2 diabetes, there has been an increase in HCC. Subsequently, the incidence of HCC has been rising exponentially worldwide over the past 20 years. Efforts to understand the molecular mechanisms behind the development of HCC have pointed to ILK as being an important player.

ILK has been implicated in various different cancers as being a useful prognostic marker, with expression correlating with tumor stage and patient survival^{33,51–53}. However, within the context of HCC, no correlation has been observed with tumor grade, and ILK expression is not standard across human patient samples, potentially suggesting different cellular mechanisms occurring simultaneously^{31,32}. Interestingly, data do support a complex role for ILK in the signaling pathway for HCC as there is a significant correlation between ILK expression and ser473 protein kinase B (PKB) phosphorylation in HCC samples^{31,32}. PKB activation and phosphorylation, of which ILK plays a role^{53–55}, have been implicated in promoting carcinogenesis in various different organs by stimulating cell proliferation and inhibiting apoptosis^{56–58}. Taken together, ILK is most likely playing a role in promoting HCC by activating PKB but should not be used to assess the tumor stage and patient survival.

Additionally, patients that present with steatosis either through nonalcoholic or alcoholic liver disease are also at an increased risk for liver cancer⁵⁹. Literature suggests that lipid accumulation in hepatocytes causes changes to the ECM and supports tumor growth and that aberrant Wnt signaling is a major player in steatosis-induced tumorigenesis^{60,61}. Interestingly, ILK is known to interact with Wnt to stimulate B-catenin expression and promote proliferation of tumor cells⁶².

CONCLUSIONS

Integrin linked kinase continues to show up in the literature as an essential player in the physiology and pathobiology of liver and other organs. Since the initial discovery of ILK 25 years ago⁴, it has proven itself to be an extremely vital protein involved in regulation of signals between cells and the ECM.

In the context of the liver, the majority of studies have focused on the role of ILK in hepatocytes, while only few have focused on nonparenchymal cells of the liver⁶³. Other functions that have been found to be linked to ILK in the liver have been nitric oxide synthase in hepatic sinusoidal endothelial cells and epithelial-to-mesenchymal transition^{64,65}. Future studies revolving around ILK and the liver should focus on its role on other hepatic cells, especially stellate cells.

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