Role of Integrin-Linked Kinase in Neuronal Cells

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Role of integrin-linked kinase in neuronal cells.

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Abstract

Integrin-linked kinase (ILK) is a focal adhesion serine/threonine protein kinase that binds to the cytoplasmic domain of β1 integrin and has an important role in integrin and growth factor signaling pathways. Clustering of integrins on the cell surface in contact with the extracellular matrix induces focal adhesion that recruits numerous mitogenic signaling proteins other than ILK, such as growth factor receptors, mitogen-activated protein kinase, and small GTP-binding proteins, to integrin receptors and forms signaling centers where adhesive and mitogenic pathways can integrate. ILK is highly expressed in neuronal cells and its enzyme activity is activated by cell adhesion on the extracellular matrix in a phosphatidylinositol 3-kinase-dependent manner. Recent studies demonstrated that ILK interacts with and regulates many different signaling pathways in neuronal cells, which implies an important role for ILK in a variety of neuronal functions. This article discusses the role of ILK in neuronal cells and also the possible involvement of ILK in neuronal disorders.

Keywords: Integrin-linked kinase, Neurite, Tau, GSK-3β, Protein kinase B/Akt, p38 MAP kinase, PI3-kinase.

Introduction

Integrin-linked kinase (ILK) is a cytoplasmic protein serine/threonine kinase that can interact with integrin β1 and β3 cytoplasmic domains [1] and has an important role in various cellular processes, such as cell adhesion, migration, growth, differentiation, and survival [2-4]. Clustering of integrins on the cell surface in contact with the extracellular matrix (ECM) induces focal adhesion [5]. ILK functions as a molecular scaffold to interact with other focal adhesion proteins such as PINCH [6], Nck-2 [7], paxillin [8], affixin [9], and calponin homology-ILK binding protein [10] at cell-ECM adhesion sites and transduces signals derived from cell-ECM interactions or other stimuli, leading to diverse cellular responses to proper signaling pathways via the formation of specific complexes with those focal adhesion signaling molecules. Moreover, ILK is also a critical effector in the phosphatidylinositol (PI) 3-kinase-dependent signaling pathway that is downstream from growth factor receptor activation [2]. ILK has a pleckstrin-homology (PH) domain that binds the PI3-kinase product, PI (3,4,5) triphosphate (PI(3,4,5)P3), and its enzyme activity is regulated in a PI3-kinase-dependent manner [11]. The protein-protein interactions at the focal adhesion site provide a framework for the formation of ILK signaling complexes that couple integrins and growth-factor receptors to the cytoskeleton and downstream kinases regulating various cellular processes [12, 13]. ILK, however, does not always transmit signals derived from extracellular events such as cell-cell and cell-ECM adhesion to the inside of the cells via integrin. Alterations in the level of ILK activity or its expression induced by various cellular responses also have a great influence on cell-cell and cell-ECM adhesion [3], suggesting that ILK can transmit signals derived from intracellular events to the outside of the cells.

Thus, ILK is involved in signal transmission of bi-directional (i.e., “from inside to outside” and “outside to inside”) transmembrane signaling pathways.

The intracellular signaling cascades that are activated when integrins bind to their ECM ligands vary depending on the cell-type [5]. The specific intracellular pathways differ depending on the specific integrin/ECM ligand interaction and on the specific integrin/intracellular signal proteins coupling in different cell types. In neuronal cells, neurite formation in response to differentiation signals is strongly promoted by ECM ligands such as laminin, fibronectin, and collagen [14, 15]. ILK is expressed in neuronal cells and is involved in neuronal differentiation [16, 17]. In addition, ILK is expressed in glial cells in various brain regions and those glial ILK proteins are involved in myelination in the central nervous system (CNS) [18]. In this review, I discuss the role of ILK in neuronal cells, focusing on intracellular downstream signaling of ILK, and propose the possible involvement of ILK in neuronal disorders.

Functional domains of ILK

The functional domains of ILK are shown in Fig.1. ILK contains four ankyrin repeats at the N-terminus followed by a PH-like domain. The N-terminal ankyrin repeats interact with the five LIM domains-only adaptor protein, PINCH, through the first LIM domain [3, 6]. The LIM domain of PINCH is a protein-binding motif consisting of a cysteine-rich consensus sequence of approximately 50 residues that form 2 separate zinc fingers [19]. PINCH also interacts with the Src-homology (SH) 2 /SH3 containing adaptor protein, Nck-2, through the fourth LIM domain [7]. Nck-2, via PINCH, couples ILK to growth factor receptor kinase or intracellular components of growth factor signaling pathways, such as insulin receptor substrate 1 (IRS-1)[4,6,7]. The PH-like

domain of ILK, through an interaction with PI (3, 4, 5) P3, is involved in growth factor and integrin receptor-induced PI3-kinase-dependent activation of ILK [11]. ILK interacts with the cytoplasmic domains of β1 and β3 integrins via its C-terminal domain [1]. The ILK C-terminal domain exhibits significant homology to other kinase catalytic domains and catalyzes serine/threonine phosphorylation of downstream target molecules, including β integrins, protein kinase B (PKB)/Akt, and glycogen synthase kinase (GSK)-3, which leads to modulation of cell-ECM interactions, gene expression, or cell survival [1,11,20-25]. The calponin homology domain-containing ILK binding protein (CH-ILKBP; also known as α-parvin and actopaxin), which interacts with the C-terminus of ILK, connects to the actin cytoskeleton [10]. CH-ILKBP localizes to focal adhesions and the cytoskeleton, and regulates cell adhesion, cell spreading, cell survival, and the localization of ILK to focal adhesions [10,12,13,27]. A close homology of CH-ILKBP, affixin (also known as β-parvin), and the focal adhesion protein, Paxillin, also interact with the C-terminus of ILK, connect to the actin cytoskeleton, and regulate cell spreading [8, 9].

ILK activity in neuronal cells

N1E-115 neuronal cells exhibit neurite outgrowth in response to serum deprivation [28,29]. Neurite outgrowth in serum-starved N1E-115 cells is enhanced when cells are grown on a laminin matrix [30]. On the other hand, neurite outgrowth of the pheochromocytoma (PC)12 cells, which are induced to differentiate into sympathetic neuron-like cells by growth factors such as nerve growth factor (NGF), is also affected by cell adhesion to the ECM [31,32]. Thus, the ECM is importantly involved in neurite outgrowth. Recent studies demonstrated that ILK is highly expressed in neuronal cells and also that the expression level of ILK proteins remains constant

during neuronal differentiation [16]. ILK activity in N1E-115 cells is transiently activated after seeding on a laminin matrix under serum-free conditions [16]. Moreover, transient ILK activation is also observed in PC12 cells grown on laminin or collagen after NGF treatment [17]. Thus, ─ transient activation of ILK occurs in both integrin- and growth factor-mediated neuronal differentiation. ILK activation under neuron-differentiating conditions is prevented by treatment of the cells with LY294002, a specific PI3-kinase inhibitor [16], suggesting that ILK activation is PI3-kinase-dependent in neuronal cells, similar to other cells [11,33]. On the other hand, weak basal ILK activity is also detected in those neuronal cells, even under non-differentiating conditions [16,17]. Such weak basal activity of ILK, therefore, might be necessary for maintaining neuronal activity. The mechanism that maintains basal ILK activity, however, remains unknown. A recent study using integrin-activating peptide indicated basal activities of both ILK and PI3 kinase in cultured hippocampal neurons and transient upregulation of ILK following integrin activation without further increase in PI3-kinase activity [24]. The data suggest that basal activity of PI3-kinase is necessary for basal ILK activity, which probably facilitates the full activation of ILK after integrin activation.

ILK controls both integrin- and growth factor-dependent neurite outgrowth

A stable transfection of a kinase-deficient mutant of ILK (DN-ILK) results in inhibition of integrin-dependent neurite outgrowth in N1E-115 neuroblastoma cells [16]. ILK activity in the parental cells was transiently activated after seeding on the laminin matrix under serum-free conditions, whereas that in the DN-ILK-transfected cells was not [16]. These results suggest that transient activation of ILK is required for integrin-dependent neurite outgrowth. Under the same

conditions, p38 mitogen-activated protein (MAP) kinase, but not MAP kinase/extracellular signal-regulated kinase kinase or extracellular signal-regulated kinases, was transiently activated after N1E-115 cell attachment to laminin, but not in the DN-ILK-expressed cells [16]. The time course of p38 MAP kinase activation was very similar to that of ILK activation. Furthermore, a p38 MAP kinase inhibitor, SB203580, blocked neurite outgrowth. Thus, activation of p38 MAP kinase is involved in ILK-mediated signal transduction leading to integrin-dependent neurite outgrowth in N1E-115 cells. p38 MAP kinase is important for neurite outgrowth [34-37]. The mechanism by which ILK triggers p38 MAP kinase activation after adhesion to laminin under serum-free conditions, however, is unknown. ILK interacts with PINCH, an adaptor protein comprising five LIM domains, through the amino-terminal ankyrin repeat domain [6]. PINCH interacts with the SH2/SH3 domain-containing adaptor protein Nck-2, which associates with ligand-activated growth factor receptor kinases and intracellular components of growth factor signaling pathways such as insulin receptor substrate [4,7]. Thus, the ILK-PINCH interaction seems to be critical for th formation of signal complex coupling integrins and growth factor receptors. On the other hand, p21-activated kinase (Pak), a family of Ser/Thr kinases, activates p38 MAP kinase [38]. Pak also potentially mediates Rac/Cdc42 signaling, which can lead to activation of p38 MAP kinase via Pak [39]. Nck-2 recognizes and activates Pak via the SH2/SH3 domain [40]. Therefore, a possible mechanism by which ILK activates p38 MAP kinase is that Pak is activated by direct interaction with Nck-2, which probably occurs following the ILK-PINCH interaction, and leads to p38 MAP kinase activation via its phosphorylation (Fig.2).

PC12 cells exhibit neurite outgrowth in response to NGF [41,42]. Activation of the extracellular signal-regulated kinases and p38 MAP kinase pathways is required for NGF-induced neuronal

differentiation in PC12 cells [34,43]. Recent study of the role of ILK in NGF-induced neurite outgrowth demonstrated that ILK is an important effector in NGF-mediated neurite outgrowth in PC12 cells and also in dorsal root ganglion neurons using a pharmacologic inhibitor of ILK (KP-392) and a DN-ILK overexpression system [17]. ILK activity was stimulated after treatment of cells with NGF and inhibition of ILK activity by overexpression of DN-ILK or treatment with KP-392 blocked NGF-induced neurite outgrowth. Moreover, NGF-induced stimulation of both Akt and GSK-3β, which reside in downstream signaling pathways of ILK, was inhibited in the presence of KP-392 or after DN-ILK transfection, suggesting that those kinases are involved in NGF-induced neurite outgrowth [17].

NGF-induced activation of MAP kinase pathways is critical for neurite outgrowth of PC12 cells [34,43]. The relation between the ILK- and MAP kinase-signaling pathways in NGF-induced neurite outgrowth, therefore, is very intriguing. Recently, Huang et al. demonstrated that paxillin, a focal adhesion adaptor protein involved in adhesion dynamics, is required for neurite outgrowth [44]. They also demonstrated that paxillin is phosphorylated by p38 MAP kinase and phosphorylated paxillin is involved in NGF-induced neurite outgrowth [44]. Moreover, activation of p38 MAP kinase is involved in ILK-mediated signal transduction and leads to integrin-dependent neurite outgrowth in N1E-115 cells [16]. Therefore, these reports suggest that phosphorylation of paxillin via p38 MAP kinase, which is activated by ILK activation, is involved in both NGF- and integrin-dependent neurite outgrowth.

ILK is involved in neuronal cell survival

Activation of integrin receptors in neurons promotes cell survival and synaptic plasticity [45-49]. Integrin signaling activates the PI3-kinase-Akt pathway [50-52], which is one of the anti-apoptotic pathways utilized by many cell types [24,52,53]. This PI3-kinase-Akt pathway is also the cell-survival signaling pathway activated by neurotropic factors and cytokines [54,55]. The kinase activity of Akt is dependent on the generation of PI (3,4,5) P3 and on activation of phosphatidylinositol-dependent kinase 1 (PDK1) and the putative phosphatidylinositol-dependent kinase 2 (PDK2), which phosphorylate Akt on Thr^{308} and Ser^{473}, respectively [56]. Phosphorylation of Akt on these residues leads to Akt activation [57] and, once activated, Akt acts to either inhibit pro-apoptotic or activate anti-apoptotic processes [54,57,58]. On the other hand, ILK can phosphorylate Akt on Ser^{473} [59] and promotes the survival of some non-neuronal cells in an Akt-dependent manner [11,26]. A recent study by Gray et al. [24] demonstrated that ILK has an important role in integrin-mediated cell survival in neurons. This study using hippocampal neurons demonstrated that ILK is upregulated following integrin activation and then activates Akt, which is essential for integrin-mediated cell-survival signaling in hippocampal neurons [24]. On the other hand, although PI3-kinase activity was necessary for Akt activation in neurons, it was not upregulated following integrin activation [24]. Thus, ILK functions as a transducer of survival signaling initiated by integrin receptor activation mainly via Akt activation, which is necessary for basal PI3-kinase activity, but not for its upregulation.

Role of ILK in oligodendrocytes

In the brain, ILK is expressed not only in neuronal cells, but also in glial cells and Purkinje cells [18]. Oligodendrocytes in theCNS, similar to Schwann cells in the peripheral nervous system,

have an important role in the formation of myelin through extending large lamellae to spiral around the adjacent axons [18,60-62]. Recent studies demonstrated that laminin-2 (LN-2; also called merosin) stimulates oligodendrocytes to extend elaborate membrane sheets through integrin α6β1 in vitro [61]. LN-2 is expressed on the axonal surface in the developing CNS [63] and congenital LN-2 deficiency is associated with abnormal CNS white matter [64]. Examination and analysis of the role of LN-2 in CNS myelination using LN-2-deficient mice demonstrated that ILK is necessary for the LN-2-induced cell spreading and myelin formation in oligodendrocytes [18]. ILK is expressed in oligodendrocytes and LN-2 stimulates ILK activity [18]. Moreover, ILK expression during early myelination in vivo is restricted to interfascicular oligodendrocytes and is only detected from postnatal day 9, which is when myelination occurs [18]. These studies suggest that ILK activation is an initial step in the intracellular signaling cascade of LN-2-induced oligodendrocyte cell spreading and myelination after cell adhesion on LN-2 via integrin. LN-2 activated Akt, activation of which is regulated via phosphorylation at two sites, Thr-308 and Ser-473, by PDK-1 [56] and ILK [59] or PDK-2 [56], respectively, continuously up to 6 h during LN-2-induced cell spreading [18]. On the other hand, DN-Akt-transfected cells had decreased LN-2-induced cell spreading, suggesting that Akt activation is involved in LN-2-enhanced cell spreading in oligodendrocytes [18]. Moreover, DN-ILK blocked phosphorylation of Akt Ser^{473} and also of GSK-3β Ser^{9}, which is a downstream substrate of ILK and Akt [11,20,65], and inhibited LN-2-induced cell spreading and myelination [18]. These results suggest that ILK-downstream signaling molecules, Akt and GSK-3β, are critical for LN-2-induced cell spreading and myelination in oligodendrocytes.

Relevance of ILK to neuronal disorders

Tau is a microtubule-associated protein that stabilizes microtubules within neurites and axons [66]. It is hypothesized that tau hyperphosphorylation leads to the destabilization of microtubules and aggregation of tau proteins, which impairs axonal transport and eventually results in neuronal cell death [66-68]. Indeed, tau hyperphosphorylation appears to be an early event preceding the formation of paired helical filaments (PHF) in the brains of patients with Alzheimer's disease [69]. On the other hand, tau phosphorylation also seems to control microtubule dynamics during neurite outgrowth and neuronal maturation, because embryonic and neonatal tau is much more heavily phosphorylated than adult tau [70-73]. Recently, Ishii et al. [74] demonstrated that stable transfection of DN-ILK to inactivate endogenous ILK results in aberrant tau phosphorylation in N1E-115 cells at sites recognized by the Tau-1 antibody (recognizes tau dephosphorylated at Ser^{195}, Ser^{198}, Ser^{199}, Ser^{202}, and Thr^{205} [75,76]) and also the anti-phospho(Ser^{199}, Ser^{202})-Tau antibody (recognizes tau aberrantly phosphorylated at Ser^{199} and Ser^{202}), which are identical to some of the phosphorylation sites in PHF-tau in the brains of patients with Alzheimer's disease [77,78]. These results suggest that inactivation of endogenous ILK results in aberrant hyperphosphorylation of tau. On the other hand, tau in the non-transfected parental cells was not phosphorylated at sites recognized by those antibodies under both normal and differentiating conditions, suggesting that endogenous ILK prevents aberrant tau phosphorylation [74]. Similarly, treatment of PC12 cells with KP-393, a selective ILK inhibitor, resulted in aberrant hyperphosphorylation of tau, which was recognized by antibody PHF-1 (recognizes tau phosphorylated at Ser^{396} and Ser^{404}) [17]. Immunofluorescence staining of cell monolayers with the antibody against aberrantly phosphorylated tau revealed that

DN-ILK-transfected cells, but not parental cells, were strongly stained with the antibody against phosphorylated tau under normal and differentiating conditions [74]. Aberrantly phosphorylated tau at Ser^{199} and Ser^{202} was also observed in the intracellular cytoplasm, but not in the nucleus, and participates in the formation of microtubule-like structures in the DN-ILK-transfected cells [74]. Further analysis of the DN-ILK-transfected cells using confocal laser scanning microscopy demonstrated that microtubule-like structures spread immediately under the entire plasma membrane of the cells and form basket-like structures [74](Fig. 3).

Recent studies of signal pathways involved in aberrant tau phosphorylation indicated that ILK inactivation leads to activation of GSK-3β [17,74] and inhibition of Akt [17]. GSK-3β is one of the candidate kinases that phosphorylates tau at both Ser^{199} and Ser^{202} [79] and also has an important role in the ILK-mediated signal pathway [11,20,65]. Activation of GSK-3β is dependent on Tyr^{216} phosphorylation [80]. On the other hand, GSK-3β activity is inhibited by direct phosphorylation at Ser^{9} by ILK [20,21] and by PKB/Akt, which is also activated via phosphorylation at Ser^{473} by ILK [11,81]. Interestingly, Tyr^{216} in GSK-3β is highly phosphorylated in DN-ILK-transfected neuronal cells, but very weakly phosphorylated in parental cells [74]. In contrast, Ser^{9} in GSK-3β was highly phosphorylated in parental cells but not in DN-ILK transfected neuronal cells [74]. Moreover, these phosphorylation levels were not significantly different between non-differentiating and differentiating conditions. These studies suggest that ILK inactivation results in Ser^{9} dephosphorylation and increased Tyr^{216} phosphorylation in GSK-3β, thereby activating the enzyme. The specific tyrosine kinase, which might be activated by ILK inactivation, however, is not known. To understand the ILK-mediated

regulatory mechanisms of GSK-3β, the specific tyrosine kinase involved in GSK-3β Tyr^{216} phosphorylation must be determined.

Lithium is an un-competitive GSK-3β inhibitor [82]. Treatment of DN-ILK-transfected cells with LiCl prevents aberrant tau phosphorylation in a dose-dependent manner [74]. This result suggests that GSK-3β activation induced by ILK inactivation is directly involved in aberrant tau phosphorylation. Moreover, treatment of DN-ILK-transfected cells with LiCl partially recovered neurite outgrowth from its inhibition caused by inactivation of endogenous ILK [74]. These results suggest that aberrant tau phosphorylation is partly involved in the inhibition of neurite outgrowth in DN-ILK-transfected neuronal cells. Tau hyperphosphorylation decreases the association of tau with microtubules [83] and inhibits total neurite number [84-86]. Therefore, the aberrant tau phosphorylation induced by ILK inactivation might affect microtubule stability or dynamics and lead to the inhibition of neurite outgrowth. On the other hand, endogenous ILK protects tau from aberrant phosphorylation and probably maintains a kind of equilibrium status responsible for microtubule reorganization (Fig.4).

ILK-deficient mice die at the peri-implantation stage due to abnormal accumulation of actin at sites of integrin attachment at the basement membrane zone [87]. Analysis of ILK-deficient mice embryos had neuronal defects caused by impaired actin dynamics, such as shorter axonal extensions [87]. The results imply that ILK is critical for axonal extension rather than axon formation. The roles of ILK in neurite and axonal extension suggest that ILK also must have an important role in brain development and plasticity, which is closely implicated in various brain functions, such as learning and memory, in addition to involvement in neuronal disorders.

Glutamate has an important role in regulating neurite outgrowth, synaptogenesis, and cell survival in the developing CNS [88] and is a mediator of synaptic transmission and synaptic plasticity in the mature CNS [89]. Over-activation of glutamate receptors, however, is considered to be involved in the neuronal death caused by stroke, Alzheimer's disease, amyotrophic lateral sclerosis, and other neurodegenerative disorders [49,90-92]. A recent study examining integrin-mediated cell survival in hippocampal neurons demonstrated that activation of ILK and its downstream signal molecule Akt promotes neuroprotection from glutamate-induced apoptosis [24]. These reports suggest that ILK has a key role in integrin-mediated cell- survival signaling in the pathogenesis of neurodegenerative conditions in which glutamate receptors are over-activated.

Concluding remarks

This review focuses on the role of ILK in neuronal cells, especially with regard to the intracellular signal pathways, and discusses recent biochemical evidence of various ILK-mediating cellular processes to understand the potential role of ILK in neuronal cells and also the CNS. The recent evidence suggests that ILK regulates both integrin- and growth factor-mediated signaling in neurons and regulates many cellular events: regulation of microtubule stability and the rearrangement necessary for integrin- and growth factor-mediated neurite outgrowth, regulation of Akt-mediated integrin and growth factor survival signals, regulation of cell adhesion and proliferation in neurons, and regulation of integrin-mediated cell spreading and myelination in oligodendrocytes. All these functions of ILK are critical for neuronal differentiation and formation and contribute to maintenance of neuronal activity and survival, which indicates that ILK is involved in brain development and plasticity. On the other hand,

because ILK has such important roles in neuronal cells, ILK dysfunction leads to serious neuronal disorders. Although we proposed the possible involvement of ILK in neuronal disorders in this review, it might be a little early to predict neuronal diseases in which ILK is involved because there are not enough studies available. More knowledge of the ILK-signaling pathways that control neuronal activity is important for further understanding of the neuronal abnormalities caused by ILK dysfunction. Moreover, further studies to fully elucidate all of the factors that regulate ILK-signaling pathways in neuronal cells might lead to the identification of potential molecular targets for therapy of neurodegenerative diseases.

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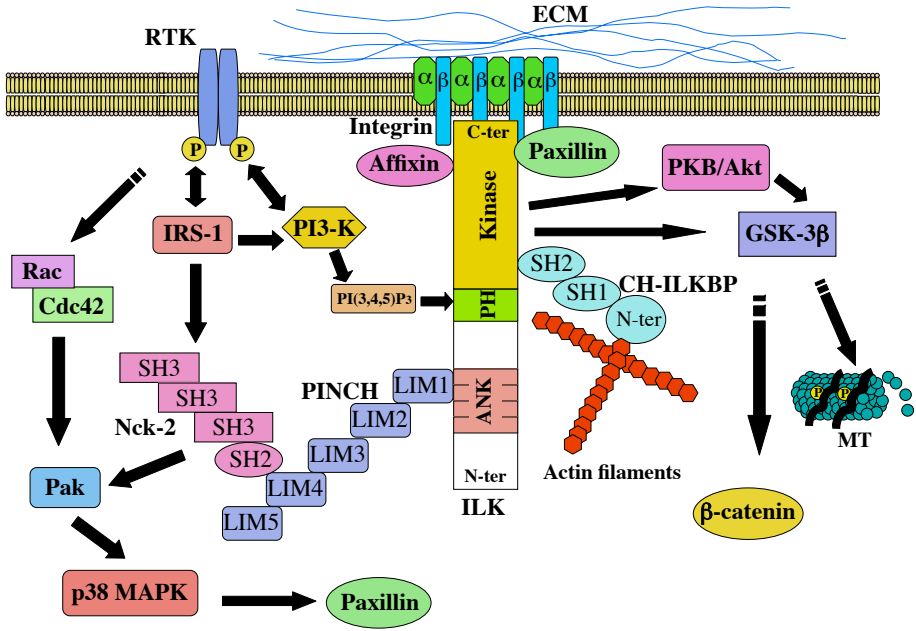
Figure legends

Figure 1. **Functional domains of ILK and hypothetical ILK-mediated signal pathways.** ECM, extracellular matrix; RTK, receptor tyrosine kinase; IRS-1, insulin receptor substrate 1; ILK, integrin-linked kinase; PKB, protein kinase B; PI3-K, phosphatidylinositol 3-kinase; PH, pleckstrin homology-like motif; GSK-3β, glycogen synthase kinase 3β; ANK, ankyrin-like repeat; SH, Src homology; LIM, LIM motif; MT, microtubule; CH-ILKBP, calponin homology domain-containing ILK binding protein; PINCH, five LIM domains-only adaptor protein; Pak, p21-activated kinase; Nck-2, Src-homology (SH)2/SH3 containing adaptor protein.

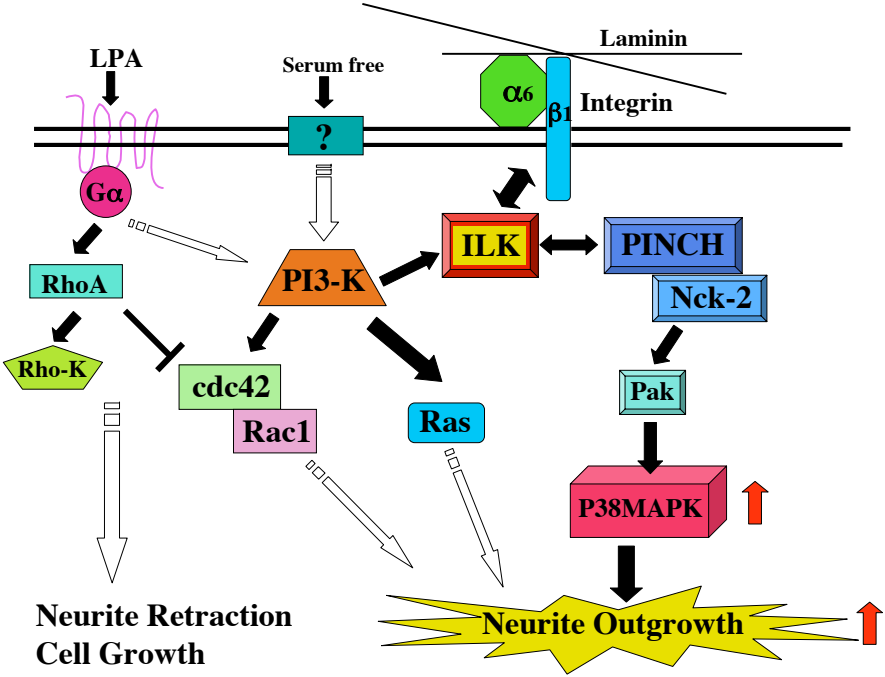
Figure 2. **P38 MAP kinase activation is involved in ILK-mediated signal transduction leading to integrin-dependent neurite outgrowth in N1E-115 cells.** ILK is activated after cell adhesion on laminin in serum-free conditions in a phosphatidylinositol 3-kinase-dependent manner. Stimulation of ILK activity results in activation of p38 MAP kinase, which is important for ILK-dependent neurite outgrowth in N1E-115 cells (16). The mechanism by which ILK triggers p38 MAP kinase activation, however, is not known. ILK can activate Nck-2 via ILK-PINCH interaction. On the other hand, Nck-2 can recognize and activate Pak. Therefore, a possible mechanism by which ILK activates p38 MAP kinase is that Pak activation by direct interaction with Nck-2, which probably occurs following the ILK-PINCH interaction, leads to p38 MAP kinase activation via its phosphorylation.

Figure 3. **Subcellular localization of aberrantly phosphorylated tau at Ser^{199} and Ser^{202}**. After cells were cultured for 16 h under either differentiating (a and b) or non-differentiating (c and d) conditions, cells were stained with anti-phospho (Ser^{199}, Ser^{202})-Tau antibody. In DN-ILK-transfected cells, the intracellular cytoplasm, except for the nucleus, was strongly stained with the antibody and microtubule-like structures were observed under normal and differentiating conditions (b and d). On the other hand, parental cells were not significantly stained under differentiating conditions (c), but very weak dot-like structures were observed in the non-differentiated cells (a). Further analysis of the DN-ILK-transfected cells using the confocal laser scanning microscopy. Microtubule-like structures spread immediately under the entire plasma membrane of the cells, and formed basket-like structures (left).

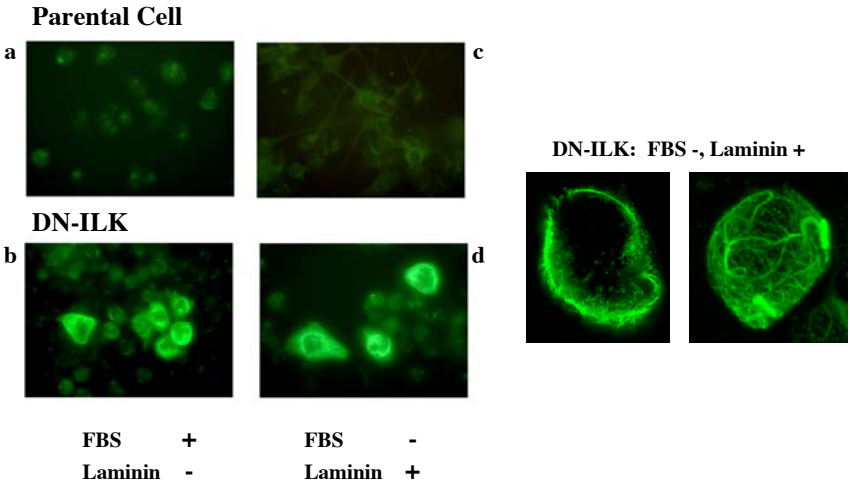
Figure 4. **Schematic model of aberrant tau phosphorylation in DN-ILK-transfected N1E-115 cells.** ILK phosphorylates GSK-3β at Ser^{9}, leading to GSK-3β inactivation. GSK-3β inactivation results in the inhibition of aberrant tau phosphorylation, an increase in microtubule stability, and induces neurite outgrowth in combination with p38 MAP kinase activation. On the other hand, ILK inactivation by DN-ILK induces GSK-3β phosphorylation at Tyr216 via activation of an unidentified tyrosine kinase, leading to GSK-3β activation. GSK-3β activation results in aberrant tau phosphorylation, microtubule instability, and decreased neurite outgrowth.



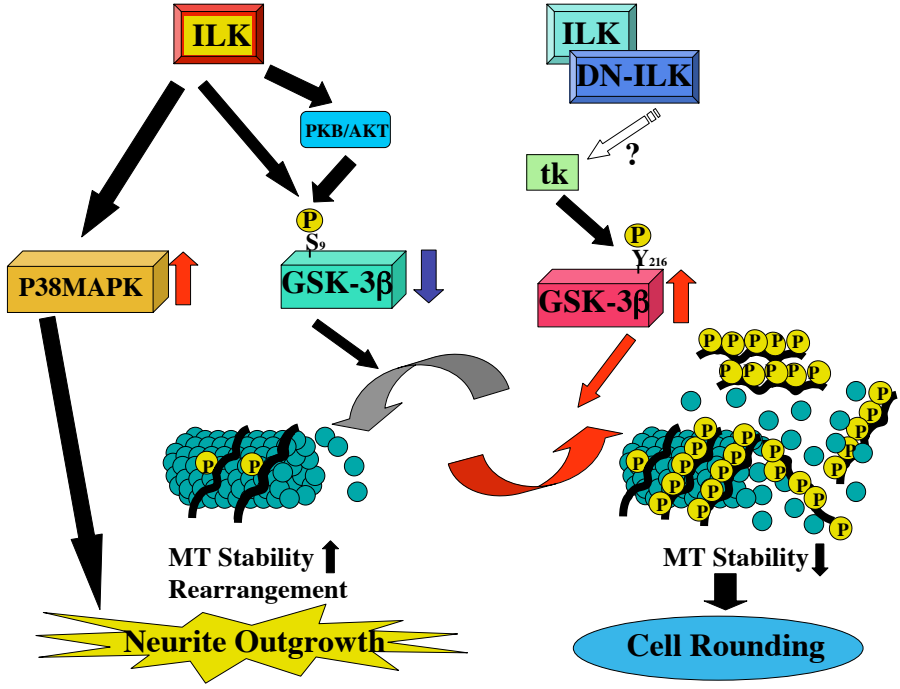
Ishii, Figure 1



Ishii, Figure 2



Ishii, Figure 3



Ishii, Figure 4