

LKB1 Regulated Pathways in Lung Cancer Invasion and Metastasis

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Abstract: Metastasis is characterized by the ability of cancer cells to invade into adjacent tissue, intravasate into blood or lymphatic vessels, and extravasate into a distant tissue. Metastatic disease is primarily responsible for the low 5-year survival rate of non-small cell lung cancer (NSCLC), and therefore, an understanding of the molecular mechanisms that regulate NSCLC metastasis is clearly warranted. The serine/threonine kinase and tumor suppressor *LKB1* is mutated in 30% of NSCLC tumors, and recent evidence points to a prominent role in NSCLC metastasis. This review summarizes LKB1-dependent invasion pathways where compromised LKB1 function could promote NSCLC metastasis.

Key Words: LKB1, Metastasis, Lung cancer, NSCLC, Adhesion, Polarity, Motility, Invasion, Anoikis, FAK, PAK1, STK11, cdc42, Energy, AMPK.

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The tumor suppressor *LKB1* is mutated in 20 to 30% of non-small cell lung cancers (NSCLCs)¹ and ranks as the third highest mutated gene in lung adenocarcinoma after p53 and Ras.^{1–3} Consequently, LKB1 has moved from a relatively understudied protein to a major player in NSCLC, especially NSCLC metastasis. Similar to other emerging pathways, the molecular details and biologic consequences of LKB1-dependent events have not been fully elucidated. The purpose of this review is to summarize LKB1 function and highlight LKB1-dependent molecular pathways that when compromised could contribute to lung cancer metastasis.

LKB1 AND LUNG METASTASIS

LKB1 is a serine/threonine kinase (formerly known as STK11)⁴ that contains two nuclear localization sequences, a central kinase domain, and a C-terminal farnesylation motif,⁵ where the N- and C-terminal noncatalytic regions share no relatedness to other proteins. LKB1 activity is regulated by the pseudokinase STE20-related kinase adaptor alpha and the scaffolding protein mouse protein 25 alpha (MO25) through a

phosphorylation-independent mechanism.^{6,7} The canonical target of LKB1 is the energy regulated AMP-activated protein kinase (AMPK), although LKB1 phosphorylates other AMPK family members such as microtubule-associated protein (MAP)/microtubule affinity-regulating kinases (MARK) 1 to 4 and Snf1-like kinases (NUAK) 1 and 2.⁸ AMPK itself is a metabolic master regulator that is activated during reduced energy availability or hypoxic stress.^{9,10} Phosphorylation of the AMPK- α activation loop at Thr¹⁷² by LKB1 is essential for AMPK catalytic activity,^{11,12} and AMPK function is compromised in *lkb1*^{-/-} mouse embryonic fibroblasts but can be restored after LKB1 reconstitution.^{13–15}

In a seminal publication, LKB1 function was assessed using a mutant k-ras-driven mouse model of lung cancer.¹⁶ In this model, *LKB1* inactivation alone was insufficient for pulmonary neoplasia, but *LKB1* inactivation in mutant k-ras tumors led to adeno, squamous, and large cell carcinomas of the lung. Importantly, these mice also had more frequent metastasis compared with tumors lacking *p53* or *Ink4a/Arf*, and increased tumor burden and larger lesions compared with k-ras mutant-only mice. Although the molecular details underlying these events were not clear, this data supported a role for *LKB1* inactivation in the progression and metastasis of K-ras-initiated lung tumors. These findings, along with its high mutation rate thrust LKB1 into the spotlight as an important regulator of lung cancer progression and metastasis. Thus, each of the following three sections summarizes how LKB1 participates in the respective metastasis-related pathway (Figure 1) and how a compromised LKB1 pathway could trigger or promote NSCLC metastasis.

CELL POLARITY AND ENERGY STRESS

In most organs, epithelial cells polarize to form an apical and basal region that provides directional transport of molecules across the epithelial sheet. LKB1 is proposed to be a master regulator of epithelial cell polarity, because LKB1 activation causes cell autonomous polarization, even in the absence of junctional cell-cell contacts.¹⁷ LKB1-induced polarization likely occurs through AMPK, because in *Drosophila*, LKB1-AMPK coordinates epithelial polarity in an energy-dependent manner,¹⁸ and in mammalian cells, AMPK regulates tight junction assembly during polarization.^{19,20} Because a loss of epithelial polarity may serve as a prerequisite for epithelial to mesenchymal transition (EMT)^{21–24} and subsequent tumor invasion,^{23,24} compromised LKB1 could trigger aberrant polarity and EMT induction. In fact,

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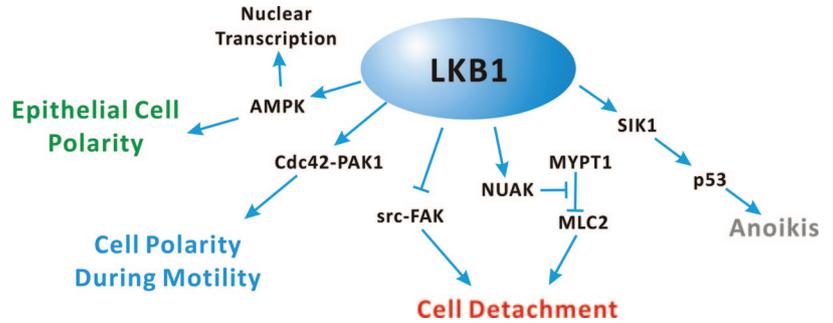


FIGURE 1. LKB1-dependent pathways related to motility and metastasis. LKB1-dependent cytoplasmic pathways that regulate epithelial cell polarity, cell polarity during motility, cell detachment/adhesion, and anoikis. For simplicity, the pathways are not shown in full.

LKB1 loss induces EMT in transformed human small airway epithelial cells,²⁵ which raises the unanswered question of whether LKB1 mutant NSCLC patients display EMT and whether this drives metastasis.

Cell polarization is also evident in migrating cells, which generate directional migration by an actin-based lamellipodia. LKB1 is necessary for lung cancer polarization during migration, where LKB1 rapidly translocates to the cellular leading edge in NSCLC cell lines to associate with actin, and regulate active cdc42 (small Rho GTPase)²⁶ through an LKB1-active cdc42-p21-activated kinase (PAK1) complex.²⁷ Loss of LKB1 activity reduces PAK1 and cdc42 activity, presumably resulting in the aberrant cell polarity observed.²⁷ Interestingly, another study in human colon cancer cell lines and mouse embryonic fibroblasts shows that LKB1 represses PAK1 by phosphorylation at a newly described Thr¹⁰⁹ site.²⁸ It is not clear why LKB1 seems to both repress and activate PAK1 function,^{27,28} although it may depend on p53 status²⁸ or whether cells are motile; nevertheless, a dysregulation of PAK1 through defective LKB1 signaling could lead to aberrant polarity and directional migration. Whether AMPK participates in these events is unclear, but AMPK also regulates mammalian cell motility and its loss causes directional migration defects,²⁹ suggesting a potential role for AMPK in energy-dependent regulation of cell motility.

It should be noted that most work on the LKB1-AMPK axis has focused on their cytosolic role, but LKB1 also functions in the nucleus,^{30–32} and more recently, stress-induced AMPK activity promotes transcription by histone 2B phosphorylation.³³ Among the potential AMPK-regulated transcripts, dual specificity phosphatases (DUSPs) may be relevant to metastasis, because the LKB1-AMPK metabolic checkpoint induces DUSP1 and 2 transcription by a p53-dependent mechanism,³⁴ and DUSPs negatively regulate mitogen-activated protein kinase (MAPK) phosphorylation.³⁵ It will be interesting to determine whether AMPK transcriptional regulation of DUSPs or other cancer relevant proteins plays a role in lung cancer metastasis.

CELL DETACHMENT AND ADHESION

Cell detachment and adhesion are necessary for motile cells to interact with the microenvironment and generate a force to move.³⁶ Primary and metastatic de novo lung cancers from mutant k-ras/lkb1 tumors show defects in cell adhesion, whereby src is activated, and focal adhesion is impaired.³⁷

Specifically, focal adhesion kinase (FAK) phosphorylation is increased in *LKB1* mutant tumors, and this correlates with increased invasion and migration. FAK is a cell-adhesion protein that signals through integrins and in some cases growth factor receptors to relay cues from the extracellular matrix (ECM) through the plasma membrane and into the cytoplasm.³⁸ There it acts as a signaling node at adhesion sites to promote cytoskeletal reorganization, adhesion, migration, and survival.^{38,39} Thus, the increased metastatic potential of mutant k-ras/lkb1 tumors could be due to stronger adhesion to the ECM, which may increase the likelihood of single cells to successfully escape the primary tumor and navigate through the microenvironment.

Interestingly, Zagorska et al.⁴⁰ suggested a potentially different mechanism for LKB1-mediated adhesion by an LKB1-NUAK1 pathway. In this case, LKB1-NUAK1 regulates cell detachment and adhesion through myosin light chain 2 and myosin phosphatase, whereby inhibition of LKB1-NUAK1 pathway impaired cell detachment and increased adhesion. This discovery is equally as exciting, and it remains to be seen whether these two LKB1-dependent adhesion pathways are linked potentially through a FAK-src-myosin light chain kinase pathway.⁴¹ In both cases, one can envision a scenario whereby LKB1 mutant tumor cells are abnormally adherent, thereby providing a mechanism for escaping cells to firmly attach to the ECM during invasion.

ANOIKIS

Anoikis is a form of apoptosis that is triggered by poor contact between the cell and the ECM. Cancer cells can become resistant to anoikis and consequently display anchorage-independent growth.^{42,43} *LKB1* participates in p53-dependent anoikis through the salt-inducible kinase (SIK1), an AMPK family member.^{8,44} SIK1 was required for LKB1 to promote p53-dependent anoikis and suppress anchorage-independent growth and invasion. SIK1 loss promoted metastatic spread and survival of cells as micrometastases in the lungs. Loss of LKB1, p53, or SIK1 resulted in anoikis resistance and, hence, survival, despite being unattached to the ECM. Thus, when taken in combination with the increased adhesion observed in LKB1 mutant cells,^{37,40} LKB1 loss could provide cells not only the ability to adhere to the ECM during invasion but also the ability to survive when unattached.

CLINICAL SIGNIFICANCE

A logical next step is to determine whether *LKB1* mutational status can be used as a predictive marker of metastatic disease. To our knowledge, a large-scale clinical study in NSCLC testing this hypothesis has not been done. Furthermore, because *LKB1* signaling negatively regulates tumor metastasis, activators of *LKB1*-dependent signaling may have clinical utility. The best characterized activator of *LKB1*/AMPK signaling is metformin, an antidiabetic drug. Several epidemiological studies show decreased cancer incidence in metformin-treated patients, and preclinical data indicated that metformin has direct antitumor effects. These works have been reviewed extensively by others.^{45–47} Metformin, however, requires *LKB1* to activate AMPK function, thus for tumors with *LKB1* inactivation, phosphatidylinositol ether lipid analogues were recently developed that can activate AMPK in *LKB1*-mutant NSCLC cells.⁴⁸ Therefore, these agents in particular could be used in *LKB1* mutant patients to “rescue” *LKB1* defects.

Taken together, *LKB1* oversees several metastasis-related pathways discussed in this review including cell adhesion, polarity, and anoikis. Many of these motility pathways are historically linked and share common signaling molecules such as FAK, myosin, and cdc42 (Figure 1). Precisely, how *LKB1* regulates these pathways and how these pathways interact are likely to be topics of interest over the next few years. Furthermore, it is unclear whether all *LKB1* mutations observed in patients or cell lines result in similar phenotypes or whether certain mutations induce pathway-specific phenotypes. In either case, a systematic evaluation of *LKB1* mutations and their effects on NSCLC invasion and metastasis is warranted. Ultimately, an understanding of *LKB1* function and how a compromised *LKB1* pathway impacts metastasis could reveal new opportunities for predicting and controlling NSCLC metastasis.

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