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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 environment. Metastatic disease is primarily responsible for the low 5-year survival rate of 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.

The tumor suppressor LKB1 is mutated in 20-30% of non-small cell lung cancers (NSCLCs)¹ and ranks as the 3rd highest mutated gene in lung adenocarcinoma after p53 and Ras¹⁻³. Consequently, LKB1 has moved from a relatively understudied protein to a major player in NSCLC, especially NSCLC metastasis. Like other emerging pathways, the molecular details and biological 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 motif⁵, where the N- and C-terminal non-catalytic regions share no relatedness to other proteins. LKB1 activity is regulated by the pseudokinase STE20-related kinase adaptor alpha (STRAD α) and the scaffolding protein mouse protein 25 alpha (MO25) through a phosphorylation-independent mechanism⁶⁻⁷. The canonical target of LKB1 is the energy regulated AMP-activated protein kinase (AMPK), though LKB1 phosphorylates other AMPK family members such as MAP/microtubule affinity-regulating kinases 1-4 and Snf1-like kinases (NUAK) 1 and 2⁸. AMPK itself is a metabolic master regulator that is activated during reduced energy availability or hypoxic stress⁹⁻¹⁰. Phosphorylation of the AMPK- α activation loop at Thr¹⁷² by LKB1 is essential for AMPK catalytic activity¹¹⁻¹² and AMPK function is compromised in *lkb1*^{-/-} mouse embryonic fibroblasts (MEFs), but can be restored following LKB1 reconstitution¹³⁻¹⁵.

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In a seminal publication, LKB1 function was assessed using a mutant k-ras driven mouse model of lung cancer¹⁶. 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 to tumors lacking p53 or Ink4a/Arf, and increased tumor burden and larger lesions compared to k-ras mutant-only mice. Though 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, since LKB1 activation causes cell autonomous polarization, even in the absence of junctional cell-cell contacts¹⁷. LKB1-induced polarization likely occurs through AMPK, since in *Drosophila*, LKB1-AMPK coordinates epithelial polarity in an energy-dependent manner¹⁸ and in mammalian cells AMPK regulates tight junction assembly during polarization¹⁹⁻²⁰. Since a loss of epithelial polarity may serve as a pre-requisite for epithelial to mesenchymal transition (EMT)²¹⁻²⁴ and subsequent tumor invasion²³⁻²⁴, compromised LKB1 could trigger aberrant polarity and EMT induction. In fact, LKB1 loss induces EMT in transformed human small airway epithelial cells²⁵, 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 via 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 GTPase²⁶ through an LKB1-active cdc42-p21 activated kinase (PAK1) complex²⁷. Loss of LKB1 activity reduces PAK1 and cdc42 activity, presumably resulting in the aberrant cell polarity observed²⁷. Interestingly, another study in human colon cancer cell lines and MEFs shows that LKB1 represses PAK1 via phosphorylation at a newly described Thr¹⁰⁹ site²⁸. It is not clear why LKB1 appears to both repress and activate PAK1 function²⁷⁻²⁸, though 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. If AMPK participates in these events is unclear, but AMPK also regulates mammalian cell motility and its loss causes directional migration defects²⁹, 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 nucleus³⁰⁻³² and more recently, stress-induced AMPK activity promotes transcription via histone 2B phosphorylation³³. Among the potential AMPK-regulated transcripts, dual specificity phosphatases (DUSPs) may be relevant to metastasis, since the LKB1-AMPK metabolic checkpoint induces DUSP1 and 2 transcription via a p53-dependent mechanism³⁴ and DUSPs negatively regulate MAPK phosphorylation³⁵. 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 move³⁶. 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 impaired³⁷. 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 cytoplasm³⁸. There it acts as a signaling node at adhesion sites to promote cytoskeletal reorganization, adhesion, migration, and survival³⁸⁻³⁹. 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 and colleagues suggested a potentially different mechanism for LKB1-mediated adhesion via an LKB1-NUAK1 pathway. In this case, LKB1-NUAK1 regulates cell detachment and adhesion through myosin light chain 2 (MLC2) and myosin phosphatase (MYPT1)⁴⁰, whereby inhibition of LKB1-NUAK1 pathway impaired cell detachment and increased adhesion. This discovery is equally as exciting and it remains to be seen if these two LKB1-dependent adhesion pathways are linked, potentially through a FAK-src-myosin light chain kinase pathway⁴¹. 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 growth⁴²⁻⁴³. 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 if 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, since 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 anti-diabetic drug. Several epidemiological studies show decreased cancer incidence in metformin-treated patients, and preclinical data indicated that metformin has direct anti-tumor effects. These works has been reviewed extensively by others⁴⁵⁻⁴⁷. Metformin, however, requires LKB1 to activate AMPK function, thus for tumors with LKB1 inactivation, phosphatidylinositol ether lipid analogues (PIA) were recently developed that can activate AMPK in LKB1-mutant NSCLC cells⁴⁸. 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 here 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 if 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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Figure 1. LKB1-dependent pathways related to motility and metastasis

This schematic summarizes 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.