LKB1 and Src: Antagonistic Regulators of Tumor Growth and Metastasis

Jill Slack-Davis,[1] John O. DaSilva,[1] and Sarah J. Parsons[1,*]
[1]Department of Microbiology and Cancer Center, University of Virginia, Charlottesville, VA 22908, USA
*Correspondence: sap@virginia.edu
DOI 10.1016/j.ccr.2010.05.016

In this issue of *Cancer Cell*, Carretero and colleagues report that Src and FAK signaling pathways are activated in lung cancers when the tumor suppressor LKB1 is deleted. These findings suggest the use of unique combinatorial therapies for treatment of lung cancers.

LKB1 is a serine/threonine kinase mutated in patients with Peutz-Jeghers syndrome. Hemizygous mutation of the gene is associated with the formation of hamartomas, most notably in the gastrointestinal (GI) tract. Individuals with this syndrome are also predisposed to the formation of a variety of tumors, including those of colon, pancreas, breast, and uterus among others (Jansen et al., 2009). Similar to humans, mice heterozygous for *Lkb1* deletion are prone to the formation of GI hamartomas and generally die from bowel obstruction prior to true tumor formation. Progression of the murine hamartomas to neoplasia requires secondary mutations, including complete loss of LKB1 function, inactivation of PTEN and p53 pathways, or activation of oncogenes such as *Kras*. Whereas loss of LKB1 or activation of K-Ras alone will form non-small cell lung cancer (NSCLC) in mouse model systems, the combination of both mutations produces more aggressive, metastatic tumors. Furthermore, patients with NSCLC having both activating K-Ras^{G12V} and loss of LKB1 function mutations suffer a poorer prognosis than do individuals with NSCLC having K-Ras^{G12V} alone. The mechanism by which LKB1 deficiency enhances tumor progression remained largely unknown.

Carretero et al. (2010) now reveal a possible means by which LKB1 influences NSCLC tumorigenesis and metastasis. Making use of the *Kras*^{G12V}/*Lkb1*$^{-/-}$ mouse model for NSCLC, the authors compared gene expression and phosphoproteome profiles between primary *Kras*^{G12V} tumors and primary *Kras*^{G12V}/*Lkb1*$^{-/-}$ tumors as well as metastatic *Kras*^{G12V}/*Lkb1*$^{-/-}$ tumors and either of the primary tumors. Loss of LKB1 in the primary tumor resulted in increased expression of genes associated with the FAK/Src and PI3K/AKT pathways. In addition to these pathways, metastatic tumors showed increases in epithelial-mesenchymal transition (EMT), stem cell, and growth factor pathways. Src has been demonstrated to affect many of the processes regulated by these pathways, including mitogenesis, cell survival, migration/metastasis, and angiogenesis/hypoxia (Ishizawar and Parsons, 2004). Indeed, Carretero et al. (2010) showed increased Src activity and FAK expression in NSCLC cells lacking LKB1, which correlated with increased migration and invasion. Importantly, targeting the PI3K/AKT, MAPK, and Src pathways in the *Kras*^{G12V}/*Lkb1*$^{-/-}$ mice significantly reduced tumor burden compared to targeting either Src alone or PI3K/AKT and MAPK, thus demonstrating the importance of these pathways in tumor progression.

LKB1 and Src in Tumorigenesis

LKB1 negatively regulates cell growth, angiogenesis, and bioenergetics through the Ser/Thr kinase AMP kinase (AMPK) under conditions of nutrient or oxygen stress, conditions often present in tumors. Upon LKB1-mediated phosphorylation, AMPK becomes activated, inducing p53-mediated cell cycle arrest, downregulating expression of genes involved in gluconeogenesis and lipogenesis, upregulating genes involved in catabolism, and inhibiting mTOR-mediated protein synthesis. These actions result in reduced cell cycle progression, cell growth, glycolysis, and angiogenesis, all features of a bona fide tumor suppressor. It is postulated that depletion of LKB1 relieves these constraints, which in the appropriate cell context results in tumorigenesis.

As Carretero and colleagues demonstrate, loss of LKB1 expression does more than relieve constraints on cell growth. The increased activity of Src and downstream signaling components as a result of LKB1 depletion provides evidence to support the active stimulation of growth regulatory pathways. One example is illustrated by the effects of LKB1 and Src on mTor-mediated protein synthesis. Src positively regulates mTOR through PI3K/Akt, whereas AMPK negatively regulates mTOR through Raptor and TSC2 (Shaw, 2009). The observation that LKB1 loss leads to increased Src activity and elevated expression of PI3K/Akt pathway components indicates that LKB1 deficiency not only relieves the negative regulation of mTOR but also promotes its activity.

Another potential node of integration for LKB1 and Src signaling is the regulation of the cell's energy metabolism. In addition to negatively regulating cell cycle progression, cell growth, and glycolosis, AMPK stimulates expression of genes involved in mitochondrial biogenesis to enhance ATP production. This is accomplished in part through phosphorylation and activation of the transcription factor, PPARγ coactivator 1α. Src, too, has been localized to the mitochondria, where evidence suggests that it regulates EGF/EGFR-mediated reduction in ATP through phosphorylation of cytochrome c oxidase II (Demory et al., 2009). Together, the loss of AMPK activity coupled with increased Src activity as a result of LKB1 depletion is expected to decrease ATP production by the electron transport system. This hypothesis is consistent with the shift to anaerobic glycolysis characteristic of highly proliferative cells.

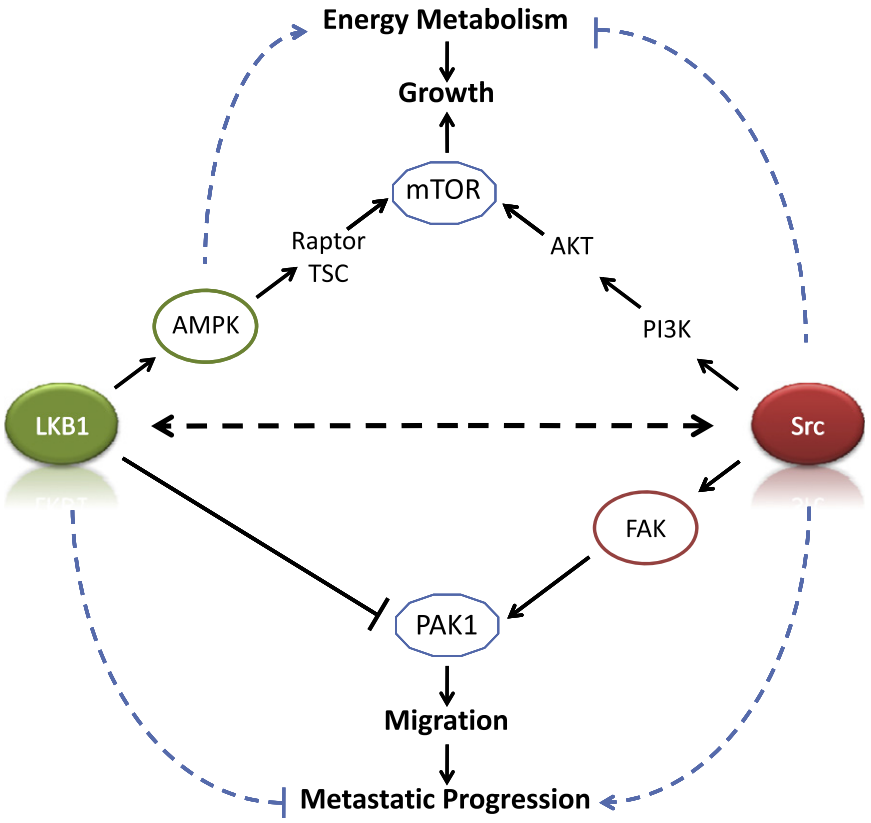

Figure 1. Antagonistic Action of LKB1 and Src Pathways Regulates Cell Growth and Migration

LKB1-induced activation of AMPK negatively modulates mTOR, whereas Src/PI3K signaling enhances mTOR function. In addition to mTOR regulation, Src and LKB1 may also regulate cell growth through the modulation of ATP production. Src and FAK promote cellular migration, in part through activation of PAK1. Conversely, LKB1 negatively regulates PAK1 activity by direct phosphorylation, reducing migration. Currently the mechanism by which LKB1 loss upregulates Src expression and activity, or whether the interaction between these pathways is unidirectional, remains unclear.

LKB1 and Src in Metastasis

A prerequisite of metastasis is increased migration. This is thought to occur as cells undergo EMT, a process that involves transcriptional upregulation of vimentin, fibronectin, TGF-β, Snail, and Twist, as well as diminished expression of E-cadherin. The EMT signature observed by Carretero et al. (2010) in metastatic $Kras^{G12V}/Lkb1^{-/-}$ tumors is difficult to ascribe purely to the loss of LKB1, because it is not observed in the primary LKB1-deficient tumors, but rather may represent a hallmark of metastasis. However, the loss of LKB1 did modulate pathways that regulate migration. Gene expression profiling demonstrated that Src, FAK, and p130Cas were elevated in $Kras^{G12V}/Lkb1^{-/-}$ tumors compared to $Kras^{G12V}$ tumors, and phosphotyrosine profiling identified FAK and paxillin, critical regulators of cellular migration.

The LKB1 and FAK/Src pathways also regulate the migratory process through their action on p21-activated kinase (PAK1). PAK1 functions downstream of the small GTPases Rac and Cdc42 to regulate adhesion turnover and actin dynamics, in part by phosphorylating paxillin (Nayal et al., 2006). FAK/Src signaling promotes PAK1 activation leading to increased migration (Slack-Davis and Parsons, 2004). Conversely, LKB1 negatively regulates PAK1 activity by direct phosphorylation of Thr109, reducing migration (Deguchi et al., 2010). Thus, with regard to PAK1 activity and cell migration, the activities of LKB1 and Src are antagonistic. It should be noted that LKB1 has been shown to positively regulate polarity of migrating cells by recruiting active Cdc42 to the leading edge to stimulate PAK1; however, the overall impact of this process on cell migration was not examined (Zhang et al., 2008).

Implications for Lung Cancer Therapy

The finding that Src/FAK pathways are involved in the progression of LKB1-deficient tumors, most notably lung, where therapeutic options are minimal, reveals new combinatorial treatment possibilities. The recent successes with small molecule and antibody inhibitors of the EGFR in subsets of NSCLC patients, as well as the development and encouraging initial testing of competitive ATP analogs for inhibition of Src and FAK, suggest that combinations of these agents might be beneficial for a larger pool of NSCLC patients, regardless of their LKB1 status. To this end, a combination of Src and EGFR inhibitors has been used in a Phase I study of advanced/metastatic NSCLC (Haura et al., 2010). Results indicate that the combination was tolerated and 62% experienced responses. However, for patients who exhibit LKB1 heterozygosity, combination of activators of AMPK and one or more tyrosine kinase inhibitors might be more efficacious (e.g., metformin with dasatinib). These strategies, molded to individual molecular signatures, hold much promise for patients with tumors harboring mutations in the LKB1 gene.

Conclusion

Taken together, this work unambiguously links Src/FAK signaling with LKB1 function and identifies novel cooperating pathways that promote the progression of lung cancers to a metastatic state (Figure 1). Further characterization of the interaction between these pathways will be of great interest. Understanding whether the loss of LKB1 function is context dependent or will generate similar signatures of pathway modulation when oncogenic progression is driven in conjunction with PTEN, p53, or B-Raf mutation instead of $K\text{-}Ras^{G12V}$ will enhance rational approaches for therapeutic intervention.

REFERENCES

Carretero, J., Shimamura, T., Rikova, K., Jackson, A.L., Wilkerson, M.D., Borgman, C.L., Buttarazzi, M.S., Sanofsky, B.A., McNamara, K.L., Brandstetter, K.A., et al. (2010). Cancer Cell *17*, this issue, 547–559.

Deguchi, A., Miyoshi, H., Kojima, Y., Okawa, K., Aoki, M., and Taketo, M.M. (2010). J. Biol. Chem., in press. Published online April 16, 2010. 10.1074/jbc.M109.079137.

Demory, M.L., Boerner, J.L., Davidson, R., Faust, W., Miyake, T., Lee, I., Huttemann, M., Douglas, R., Haddad, G., and Parsons, S.J. (2009). J. Biol. Chem. *284*, 36592–36604.

Haura, E.B., Tanvetyanon, T., Chiappori, A., Williams, C., Simon, G., Antonia, S., Gray, J., Litschauer, S., Tetteh, L., Neuger, A., et al. (2010). J. Clin. Oncol. *28*, 1387–1394.

Ishizawar, R., and Parsons, S.J. (2004). Cancer Cell *6*, 209–214.

Jansen, M., Ten Klooster, J.P., Offerhaus, G.J., and Clevers, H. (2009). Physiol. Rev. *89*, 777–798.

Nayal, A., Webb, D.J., Brown, C.M., Schaefer, E.M., Vicente-Manzanares, M., and Horwitz, A.R. (2006). J. Cell Biol. *173*, 587–589.

Shaw, R.J. (2009). Acta Physiol. (Oxf.) *196*, 65–80.

Slack-Davis, J.K., and Parsons, J.T. (2004). J. Cell. Biochem. *91*, 41–46.

Zhang, S., Schafer-Hales, K., Khuri, F.R., Zhou, W., Vertino, P.M., and Marcus, A.I. (2008). Cancer Res. *68*, 740–748.

Glycogen Synthase Kinase-3 and Leukemia: Restoring the Balance

Noah W. Birch[1] **and Nancy J. Zeleznik-Le**[1,*]
[1]Oncology Institute, Department of Medicine, Loyola University Medical Center, Maywood, IL 60153, USA
*Correspondence: nzelezn@lumc.edu
DOI 10.1016/j.ccr.2010.05.017

The role of GSK-3 in oncogenesis is paradoxical, acting as a tumor suppressor in some cancers and potentiating growth in others. In this issue of *Cancer Cell*, Wang et al. provide some mechanistic insight into GSK-3 activity's role in potentiating leukemias which are dependent on *homeobox* (*HOX*) gene misregulation.

Glycogen synthase kinase-3 (GSK-3) was first identified for its role in regulating glycogen metabolism but has since been shown to be involved in the regulation of a variety of processes including signal transduction, gene expression, and cell-fate determination (Jope and Johnson, 2004). These critical roles have become increasingly more appreciated as misregulated GSK-3 has been implicated in neurological disorders (i.e., Alzheimer's disease and bipolar disorder), non-insulin-dependent diabetes mellitus, stroke, and neoplasias (Cohen and Frame, 2001; Rayasam et al., 2009). With over 40 proteins identified as potential GSK-3 substrates, it is not surprising that a complicated network of GSK-3 functioning has emerged (Jope and Johnson, 2004). This complexity is particularly evident in cancer where GSK-3 can take on seemingly opposing roles in tumor suppression or promotion (Ougolkov and Billadeau, 2006; Luo, 2009).

Decreased expression or activity of GSK-3 has been associated with skin and breast tumors. In vitro and in vivo rescue experiments in which active GSK-3 is restored to transformed tumor-cells leads to suppression of cell proliferation. Tumor-suppressive functioning of GSK-3 has been shown to involve the WNT signaling pathway in which active GSK-3 negatively regulates β-catenin through inhibitory phosphorylation and prevents transcription of β-catenin target genes involved in cell-cycle progression (Figure 1). Decreased expression or activity of GSK-3 could, then, activate the WNT signaling pathway through stabilization of β-catenin and contribute to tumorigenesis (Luo, 2009; Rayasam et al., 2009). In contrast, overexpression of active GSK-3 is associated with increased proliferation and decreased patient survival of some cancers through pathways which are thought to involve cyclin D1 and NF-κB (Luo, 2009).

This opposing function of GSK-3 as a tumor promoter has also been suggested for acute leukemia in a report by Wang and colleagues in which MLL-associated leukemia was shown to depend on GSK-3 for sustained proliferation of transformed cells (Wang et al., 2008). Pharmacologic inhibition of GSK-3 caused decreased proliferation, reduced cell-cycle progression, and increased myeloid differentiation of leukemia cells that had been transformed with chimeric MLL oncoproteins. Decreased GSK-3 activity in MLL leukemia cells was associated with increased levels of β-catenin, decreased cell proliferation in vitro, and enhanced survival of mice with these leukemias in vivo. An increase in the CDK inhibitor, $p27^{Kip1}$, expression was observed specifically in MLL leukemia cells upon treatment with GSK-3 inhibitors suggesting that GSK-3 may override cell-cycle regulators in MLL cells to enhance proliferation, though the exact mechanism remained unclear.

In the current issue of *Cancer Cell*, Wang et al. present a follow-up of their previous study where they begin to delineate the mechanism whereby MLL leukemias depend on GSK-3 for maintenance of proliferation and transformation (Wang et al., 2010). Their data demonstrate that GSK-3 activity promotes the formation of a HOX/MEIS1/CREB complex that recruits coactivators CBP and TORC to maintain the MLL leukemia stem cell transcription program. It has been well established that *homeobox* (*HOX*) genes become misregulated in aggressive leukemias involving *MLL* translocations (Shah and Sukumar, 2010). During normal hematopoiesis, MLL maintains appropriate *HOX* gene expression in hematopoietic stem and progenitor cells. As progenitor cells mature into differentiated