IN THE SPOTLIGHT

Energizing the Search to Target *LKB1*-Mutant Tumors

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Summary: *LKB1* is the third most frequently mutated gene in non–small cell lung cancer and serves as a master regulator of cell metabolism and polarity across a variety of model organisms. Recent studies are beginning to identify therapeutics that exploit defects associated with LKB1 loss. The work presented here by Liu and colleagues shows that deoxythymidylate kinase is a new potential target in LKB1-deficient tumors and highlights the possibility of a new therapeutic option for this subset of patients with cancer. *Cancer Discov; 3(8); 843–5. ©2013 AACR.*

See related article by Liu et al., p. 870 (10).

Clinical interest in the serine/threonine kinase LKB1 first surfaced when *LKB1* mutations were identified as the cause of the autosomal dominant disorder Peutz–Jeghers syndrome, in which patients have benign gastrointestinal hamartomatous polyps and increased cancer risk later in life (1). This was followed by reports identifying somatic *LKB1* mutations in non–small cell lung cancer (NSCLC; refs. 2, 3), cervical cancer (4), and melanoma (5). Alongside these clinical discoveries, studies investigating the cellular role of LKB1 were advancing. In brief, LKB1 was shown to be a major regulator of cell metabolism and polarity (reviewed in ref. 6). During energy stress, LKB1 phosphorylates the energy sensor AMP-activated protein kinase (AMPK), which suppresses cell growth and biosynthesis to conserve ATP. LKB1 also phosphorylates 12 related AMPK subfamily members, which have additional functions including cell polarity maintenance. LKB1 loss causes defects in epithelial cell polarity and polarity during motility, and triggers the proinvasive epithelial-to-mesenchymal transition (EMT) program. Thus, in the last two decades, clinical and basic science data have thrust LKB1 into the spotlight at the crossroads of metabolism, cell polarity, and tumor progression.

Although insights into our understanding of LKB1 function in normal and cancerous tissues have gained momentum, identification of therapeutics that exploit LKB1 deficiency has lagged behind. This is especially critical in NSCLC, where most *LKB1*-mutant tumors occur in smokers who lack EGF receptor (*EGFR*) mutations or *ALK* translocations (7, 8) and therefore are not eligible for targeted therapy. *LKB1* is the third most frequently mutated gene in NSCLC, after *TP53* and *KRAS*, occurring in 20% to 30% of all adenocarcinomas (2, 3). However, unlike *EGFR* mutations, which have been successfully targeted, or *KRAS* mutations, which have not, *LKB1* mutations occur across the entire gene and are of various types. In fact, a co-clinical trial (9) showed that *Lkb1*- and *Kras*-mutant tumors were resistant to docetaxel plus a MAP–ERK kinase (MEK) inhibitor, a strategy that was effective against tumors bearing *Kras* mutations only, thereby highlighting the possibility that *KRAS, LKB1*-mutant tumors have altered therapeutic sensitivity.

In this issue, Liu and colleagues (10) describe an innovative approach that addresses these challenges by developing a synthetic lethal screen to elucidate therapeutic targets in *Kras/Lkb1*-mutant cell lines derived from their original mouse model (11). This short hairpin RNA (shRNA)-based screen identified deoxythymidylate kinase (*Dtymk*) as synthetically lethal with *Lkb1* loss. DTYMK is required for dTTP biosynthesis by catalyzing the phosphorylation of dTMP to dTDP; therefore, *Dtymk* depletion reduces the dTDP pool and increases the dTMP pool. Consistent with this finding, a subsequent metabolomics approach confirmed that *Lkb1*-mutant cell lines had a global reduction in metabolites involved in dTTP synthesis.

From a cell viability perspective, *Dtymk* depletion significantly reduced mouse *Lkb1*-null cell growth but left cell growth largely intact in *Lkb1*–wild-type cell lines. This could be rescued by exogenous application of dTTP to the *Dtymk* shRNA *Lkb1* cells, further emphasizing that the differing sensitivity to *Dtymk* depletion is linked to dTTP synthesis. Interestingly, basal levels of DTYMK were lower in *Lkb1*-mutant mouse cells compared with wild-type cells, which the authors speculate may explain the synthetic lethality observed between *Lkb1* and *Dtymk*. The authors propose that the *Lkb1*-null cell lines are more dependent on the dTTP synthesis pathway, and thus cells are more sensitive to *Dtymk* loss. This is an interesting possibility, and therefore it will be important to determine whether DTYMK levels correlate with *LKB1* mutational status in patients. The authors do indeed show that patients with high DTYMK levels have worse survival than patients with lower levels; however, *LKB1* mutational status was not assessed.

A next step would be to determine the *in vivo* synthetic lethality of *Dtymk* in their *Kras/Lkb1*-mutant mouse model, whereby *Dtymk* shRNA could be delivered within a lentiviral-Cre vector and metabolites in the dTTP synthesis pathway could be assessed. The authors do show that implantation of *Lkb1*-null cell lines transduced with *Dtymk* shRNA into athymic nude mice produces smaller tumors compared with

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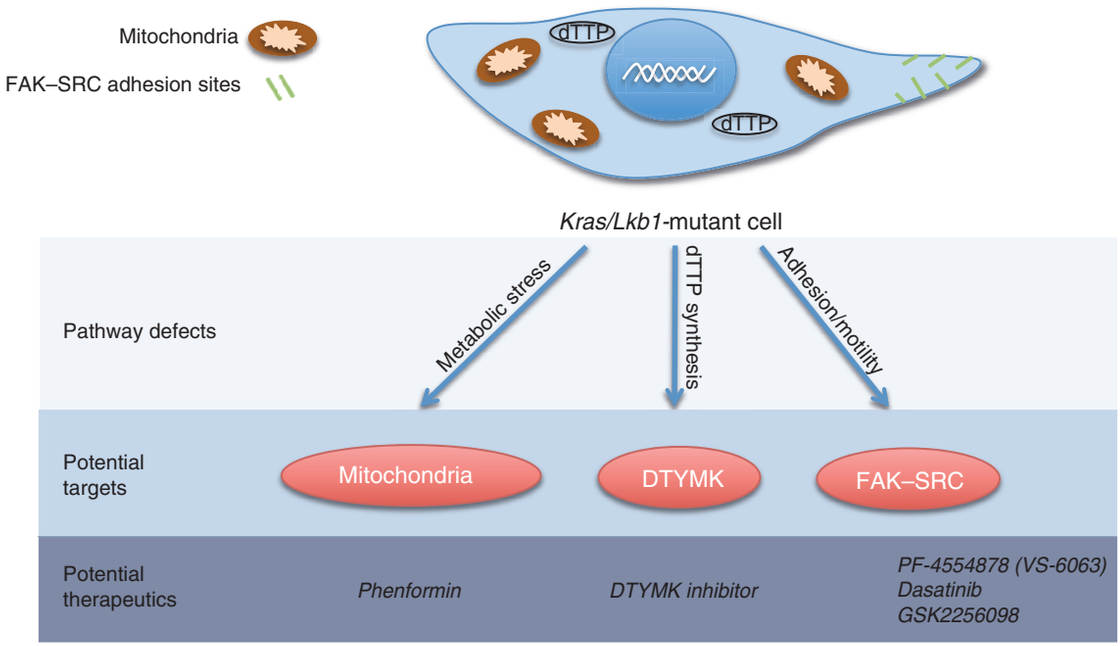

Figure 1. Diagram showing some of the pathway defects associated with a *Kras/Lkb1* mutation followed by potential targets and therapies. To our knowledge, a DTYMK inhibitor has not yet been developed against LKB1-deficient cells or tumors. FAK, focal adhesion kinase.

tumors from *Lkb1*–wild-type cells, though metabolic analysis was not conducted to assess defects in the dTTP pathway.

The Shaw lab at the Salk Institute (co-authors here as well; San Diego, CA) has also begun to tackle how LKB1-deficient tumors can be exploited (co-authors here as well) and recently showed that LKB1-inactivated cells are hypersensitive to the metabolic drug phenformin (12), which is a mitochondrial inhibitor and analog of metformin. This group reasoned that LKB1-deficient tumors would be more sensitive to metabolic stress, as they lack an active AMPK sensor and therefore cannot restore energy homeostasis. Based upon their efficacy studies, this seems to be the case, as *Kras/Lkb1*-mutant mouse tumors showed greater sensitivity to phenformin than *Kras/p53* mutants. Thus, phenformin treatment represents a new therapeutic opportunity in LKB1-deficient tumors.

Other potential targets for exploiting LKB1-deficient tumors exist. For example, *Kras/Lkb1*-mutant metastases have increased focal adhesion kinase (FAK) and SRC activation (13). Similarly, FAK hyperactivation is observed using *in vitro* models of LKB1 depletion, whereby cells have rapid focal adhesion site turnover and change directions often when motile (14). A combination of SRC, phosphoinositide 3-kinase, and MEK inhibitors led to synergistic *Kras/Lkb1*-mutant tumor regression, highlighting the possibility of this therapeutic combination. As FAK and Src play prominent roles in metastasis, it will be interesting to determine whether novel antimetastatics (e.g., FAK inhibitors) can be developed to target LKB1-deficient tumors.

The steps taken by Liu and colleagues (10) and other groups showcase the potential for therapeutics that precisely target LKB1-deficient tumors (Fig. 1). Lung cancers that harbor *LKB1* and *KRAS* mutations represent a class of tumors that have failed to show sensitivity to conventional or targeted approaches. Janne and colleagues (15) showed that targeting *KRAS*-mutant lung cancers in the clinic with docetaxel plus a MEK inhibitor was considerably more effective than docetaxel alone. The exception was in patients whose tumors contained *LKB1* and *KRAS* mutations, in which no efficacy was observed. Ramalingam and colleagues (16) showed preliminary evidence from a randomized phase II trial that targeting *KRAS*-mutant lung cancers with docetaxel plus ganetespib, a second-generation HSP90 inhibitor, seems superior to docetaxel alone, although the *LKB1* mutational status of the tumors is unknown at this time. Thus, as the therapeutic armamentarium against *KRAS*-mutant lung cancers begins to take shape, definitive approaches to targeting *LKB1*-mutant tumors remain uncertain. It is unclear whether the resistance observed in *LKB1*-mutant tumors is due to the inherent survival advantage over wild-type tumors, altered metabolic regulation, and/or prometastatic signaling such as the dysregulation of FAK–SRC. Furthermore, it is also unclear how cell polarity defects observed in *Lkb1*-mutant cells affect these events, as cell polarity is directly tied to metastasis, EMT, and metabolism. Nevertheless, the findings by Liu and colleagues (10) represent a clear opportunity to rationally develop a small molecule against DTYMK in *KRAS/LKB1*-mutant tumors. This is an approach that seems timely, biologically challenging, and clinically intriguing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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