Liver Kinase B1 (LKB1) Loss Has its p-ERKS: ERK Inactivation as a Vulnerability in NSCLC With LKB1 Mutations

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Over the previous decade, cancer field has experienced an unprecedented revolution driven by next-generation sequencing studies that have cataloged the plethora of somatic genomic alterations in multiple tumor types. In NSCLC, some of these large, collaborative efforts have detailed the genomic landscape to illustrate the high molecular complexity and heterogeneity that characterize the largest subtype of lung cancer.1,2 Notably, these studies have enormously expanded our previous knowledge about the potential oncogenic drivers in lung cancer as genetic alterations such as mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, discoidin domain receptor tyrosine kinase 2, kelch like ECH-associated protein 1, neurofibromin 1, and ataxia telangiectasia mutated; genomic rearrangements in anaplastic lymphoma kinase, ROS proto-oncogene 1, or rearranged during transfection; or amplifications in nuclear factor, erythroid 2 like 2 or MYC Proto-Oncogene, BHLH Transcription Factor have been discovered in addition to previously known mutations in oncogenes (i.e., Kirsten rat sarcoma 2 viral oncogene homolog [KRAS], v-Raf Murine Sarcoma Viral Oncogene Homolog B, or EGFR) and tumor suppressor genes (TSGs) (i.e., tumor protein 53 or liver kinase B1 [LKB1]). These findings provided the rationale for the development of new targeted agents, and as a consequence, the clinical armamentarium tremendously expanded from that on the basis of conventional chemotherapy. In this context, targeted agents against various oncogenes such as EGFR, v-Raf Murine Sarcoma Viral Oncogene Homolog B, anaplastic lymphoma kinase, or ROS proto-oncogene 1 are now being used in clinical practice.3 Nonetheless, the actual implications of genomic alterations in TSGs within the framework of patient treatment are largely unknown. Therefore, circumscripting the potential role of such genetic alterations to existing targeted therapies would offer new opportunities for therapeutic intervention in patients with NSCLC.

One of the most typically mutated TSGs in NSCLC, in up to 30% of patients, is LKB1, alternatively known as serine threonine kinase 11. Mutations in LKB1 were originally discovered in individuals with the autosomal dominant Peutz-Jeghers syndrome.4 Individuals with these germline mutations have an increased risk of cancer development compared with the general population, including a higher risk of lung cancer.5,6 Missense mutations in LKB1 abrogate its kinase function, impairing autophosphorylation7 or direct phosphorylation of substrates such as 5’ adenosine monophosphate-activated protein kinase (AMPK).8 In 2002, the seminal work by Sanchez-Cespedes et al.9 described LKB1 mutations as a common event in NSCLC. LKB1 mutations are characteristic to patients with NSCLC with a history of smoking.10 Consequently, concurrent mutations with the KRAS oncogene but without EGFR are found in a large percentage of cases. Patients with NSCLC with concurrent mutations in LKB1 and KRAS display a worse prognosis compared with patients with KRAS mutation and coexisting mutations in tumor protein 53.11 Moreover, LKB1 mutations drive primary resistance against the inhibitors of the programmed cell death protein 1 (PD-1) axis in...
patients harboring KRAS mutations. Overall, these findings highlight the need for novel personalized strategies for the treatment of patients with NSCLC with compromised LKB1 function.

This issue of Journal of Thoracic Oncology features preclinical data from Caiola et al. that illustrate the antitumor efficacy of the ERK inhibitors (ERKis) SCH772984 and ulixertinib in NSCLC, in which normal LKB1 expression is lost. First, screening of antitumor drugs revealed that ERKis negatively affected the viability of an NSCLC cell line mutant for LKB1, suggesting that the mutational status of this TSG could be driving sensitivity to the target agent. To formally address this question, the investigators deployed complementary loss- and gain-of-function experiments in NSCLC cell lines to reveal, on the one hand, that ERKi-resistant, wild-type LKB1 cells became sensitive to the inhibitor on genetic abrogation of LKB1 expression and, on the other hand, that LKB1-deficient cell lines became insensitive to ERKis after LKB1 reconstitution. Notably, sensitivity to ERKis was recapitulated in LKB1-deficient NSCLC cells expressing the KRAS oncogene. More importantly, the specific antitumor effect of ERKi was mirrored in vivo using LKB1-deficient NSCLC cell–derived xenografts and a genetically engineered mouse model of lung cancer driven by mutant Kras and Lkb1 deletions (Kras^L525G12D and Lkb1^lox/lox respectively). These current findings describing the exquisite sensitivity of LKB1-deficient cells to ERKis are in tune with those of previous studies reporting the specific antitumor activity of MEK inhibitors in LKB1-mutated NSCLC cells compared with that in cells with a native allele and, overall, suggest that the MEK-ERK axis represents an actionable vulnerability in this subgroup of patients with NSCLC.

To query the underlying molecular mechanisms involved in the differential sensitivity of LKB1-proficient and -deficient cells to ERK inhibition, changes in the signaling proteins of key effector pathways were analyzed by Western blot. The investigators found that the activation of S6 ribosomal protein was preferentially abrogated in LKB1-deficient cells compared with that in wild-type counterparts because of the compensatory activation of AKT and recovery of ERK activation at 12 hours after inhibitor treatment. Additional protein analyses revealed the upstream inactivation of 90 kDa ribosomal protein S6 kinase 1 or p90RSK1, a mechanism likely to be independent of ERK activation. Notably, the genetic deletion of 90 kDa ribosomal protein S6 kinase 1 or p90RSK1 in wild-type LKB1 NSCLC cells abrogated S6 phosphorylation after exposure to the ERKi, mimicking the LKB1-lost phenotype. While trying to extend the mechanistic data to NSCLC cells with concurrent mutations in LKB1 and KRAS, the investigators observed that activating mutations in the PI3K pathway conferred resistance to LKB1-mutated cells. In this regard, the investigators acknowledged the limitations of treating LKB1-deficient cells using an ERKi and provided additional in vitro and in vivo data supporting the combined administration of ERKi and PI3K inhibitor to circumvent this mechanism of resistance.

Collectively, the study by Caiola et al. provides substantial preclinical data supporting the use of ERKis for the treatment of NSCLC, in which LKB1 is mutated. A limitation of the study resides in the fact that most experiments were carried out in an NSCLC cell line, in which LKB1 was abrogated or reconstituted by genetic manipulation. In this regard, analysis of a large panel of cell lines with spontaneous somatic LKB1 loss featuring the ample spectrum of mutations found in NSCLC will be mandatory to confirm the interesting data described in this study. This complementary analysis will enable a deeper characterization of the sensitivity of LKB1-deficient NSCLC cells to ERKis not only in the context of concurrent mutations in oncogenes but also in the context of additionally mutated TSGs.

In addition, the findings by Caiola et al. pose a series of questions that are yet to be addressed. First, a side-by-side comparison about the efficacy and, more importantly, the toxicity of ERKis to that of MEK inhibitors remains to be performed. This becomes an important issue in the context of the combinatorial therapeutic strategies proposed by the investigators because concomitant MEK and PI3K inhibition have led to severe toxicity profiles in patients with cancer that could, in addition, be anticipated with the use of ERKis. Second, despite the significant antitumoral effect of ERKi, no tumor regression was observed in the different in vivo models described in the featured research article, suggesting that the administration of additional pharmacologic inhibitors will be required to effectively treat NSCLC with LKB1 loss. LKB1-mutated NSCLC has been previously shown to be sensitive to the metabolic drug phenformin. Thus, preclinical testing of ERKi and phenformin combination, especially because different pathways are targeted by both these drugs, stands as a potential therapeutic option to be considered in this NSCLC subgroup. Finally, mutations in LKB1 features a subgroup of NSCLC with the expression of the KRAS oncogene largely refractory to the immune-checkpoint inhibitors anti–PD-1 and anti–programmed death ligand 1. Interestingly, in the preclinical models of mutant Kras-driven NSCLC, MEKis remodel the immune environment to enhance the efficacy of anti–PD-1 and anti–programmed death ligand 1. Therefore, assessing the efficacy of ERKis in combination with immune-checkpoint inhibitors will require further attention as it may positively impact survival of patients with NSCLC with LKB1 mutation.

Given the specific effect of ERK inactivation in NSCLC with dysfunctional LKB1, defining the group of patients...
with NSCLC who would benefit from this interventional approach arises as a question of paramount importance. Somatic mutations in LKB1 include insertions, deletions (including large intragenic deletions); nonsense, frame-shift, and missense mutations; splice site alterations; and, although less frequent, promoter hypermethylations. In this regard, immunohistochemistry has been proposed as the most accurate method to define patients with a loss of LKB1 expression. In addition, LKB1 activity in NSCLC can be compromised beyond somatic mutations. As an example, aurora kinase A–mediated LKB1 phosphorylation impairs AMPK activation, exacerbating the cancerous NSCLC phenotype. Furthermore, LKB1 acts as a master kinase activating various AMPK-related kinases (brain-selective kinase 1, MAP/microtubule affinity-regulating kinase 1, NUAK family SNF1-like kinase 1, and salt-inducible kinase 1) to control multiple cellular functions potentially involved in tumor suppression.

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References