IN THE SPOTLIGHT

Evading the STING: LKB1 Loss Leads to STING Silencing and Immune Escape in KRAS-Mutant Lung Cancers

Carminia Maria Della Corte and Lauren Averett Byers

Summary: Mutations in STK11 (LKB1) are a major cause of primary resistance to immunotherapy in non–small cell lung cancer. Kitajima and colleagues dissect the underlying mechanism of this immune-resistant phenotype, demonstrating that LKB1 loss leads directly to suppression of stimulator of interferon genes (STING) and insensitivity to cytoplasmic double-strand DNA detection. Therapies that reactivate LKB1 or the STING pathway may boost anticancer immune response in cancers with resistance to immune-checkpoint blockade.

See related article by Kitajima et al., p. 34 (6).

Lung adenocarcinoma accounts for the majority of non–small cell lung cancers (NSCLC) and includes a variety of molecular subtypes with distinct therapeutic vulnerabilities and resistance patterns. The most frequent oncogenic driver in lung adenocarcinoma is KRAS mutation, which is found in 25% to 30% of cases. However, previous attempts to develop KRAS-targeted therapy have failed, in large part because of their heterogeneity—a consequence of concomitant co-mutations.

In a previous landmark study led by Skoulidis and colleagues, our group described molecular features of two key subgroups of KRAS-driven lung adenocarcinoma, those with comutations in TP53 (KP) or in STK11/LKB1 (KL; ref. 1). The KP and KL subtypes were especially different in terms of metabolic and immune profiles, with KL tumors characterized by downregulation of PD-L1 and other immune-checkpoint genes (Fig. 1A).

LKB1 acts as a critical regulator of cellular metabolism and energy sensing by activating AMP kinase (AMPK). Once activated, AMPK inhibits mTOR signaling. Loss of LKB1 increases serine utilization and synthesis of S-adenosyl methionine (SAM), a substrate of DNMT1, EZH2, and other epigenetic silencing enzymes (2). Thus, STK11 mutation (and the consequent LKB1 loss) directly induces epigenetic changes and confers a proliferative advantage to cancer cells.

Among lung adenocarcinomas, STK11-mutant tumors are “immune-cold” (Fig. 1A), having low or absent PD-L1 levels, ineffective T-cell infiltration, and preferential production of myeloid-recruiting chemokines, such as IL6 (1, 3). Earlier this year, Skoulidis and colleagues further demonstrated that STK11/LKB1 inactivation is a major genetic driver of primary resistance to anti–PD-1 agents (4), and that patients harboring STK11/LKB1 alterations have shorter progression-free and overall survival after anti–PD-1 treatment (Fig. 1B). This finding was further validated by Kunkel and colleagues, who showed that STK11/LKB1 mutation in nonsquamous NSCLC predicted worse outcomes after treatment with anti–PD-L1 (durvalumab) plus/minus anti-CTLA4 (tremelimumab) from two clinical studies of previously treated patients with NSCLC (5). Together, these data strongly support STK11/LKB1 alterations as a marker of immune-checkpoint blockade resistance. However, a better understanding of the mechanisms regulating immune suppression in KL lung adenocarcinomas is critical for developing effective combination strategies that reestablish antitumor immunity.

In this issue, Kitajima and colleagues explore the mechanism by which STK11/LKB1 inactivation leads to immune escape (6). They find that LKB1 loss results in insensitivity to cytoplasmic double-strand DNA (dsDNA) detection through transcriptional silencing of stimulator of interferon genes (STING; encoded by TMEM173). The STING pathway is a critical component of the innate immune system and is responsible for sensing cytosolic DNA from pathogens (e.g., viruses or bacteria) or our own cells (e.g., cancer). In cancer cells, STING is activated by dsDNA released from the nucleus and mitochondrial compartments into the cytosol, and represents a key, intrinsic tumor-sensing mechanism.

Using gene-expression data to compare KL and KP lung adenocarcinomas, the authors found that KL lung adenocarcinomas from The Cancer Genome Atlas (TCGA) cohort and cell line models exhibit downregulation of the type I IFN signaling signature, including the TMEM173 (STING) gene (a common upstream regulator of type I IFN). They also observed upregulation of oxidative stress signaling, as expected for the mitochondrial impairment due to LKB1 dysfunction. They then validated these findings by IHC and western blot, showing that LKB1 loss at the protein level is associated with low or absent STING protein in KL tumors and cell lines (but intact CGAS levels). In contrast, KP models consistently expressed relatively higher levels of STING protein at baseline. However, expression of STING could be directly inhibited in KP models by LKB1 deletion using...
CRISPR/Cas9 silencing. Furthermore, STING expression in KL cells could be rescued by LKB1 reconstitution in cells with low levels of STING, but not in those without any detectable STING. Together, these results support the direct transcriptional regulation of STING by LKB1.

Next, the authors elegantly connected the role of LKB1 as an epigenetic regulator to their observations. By exploring the functional role of the TME173 (STING) promoter, the authors found that KL cells exhibit high activity of DNA methyltransferase DNMT1 and EZH2, which can silence STING transcription by promoter methylation. Interestingly, DNMT1 and EZH2 are both substrates of SAM, which is increased by LKB1; and treatment with specific inhibitors of DNMT1 (e.g., decitabine) and/or EZH2 restored both SAM levels and STING expression.

The authors then tested the effect of LKB1 reconstitution on DNA sensing by stimulating cells with poly (dA:dT) (which mimics cytosolic dsDNA). They found that LKB1 reconstitution in STING-/- KL cells restored STING downstream signaling. The activation of the intracellular phosphorylation cascade through TBK1 and IRF3 led to the release of the immune inflammatory cytokines IFNβ, CXCL10, CCL5, GM-CSF, CCL3, and IL1α and suppression of IL6, as well as the activation of STAT1 (which stimulates PD-L1 expression; Fig. 1C). Of note, in STING-/- KL cells, the addition of DNMT inhibitor is necessary to restore all signals, further supporting that LKB1 loss is a direct cause of suppressed tumor immunity and low PD-L1 expression. In addition, the authors confirm that transient activation of STING prompted via poly (dA:dT) and pretreatment with a DNMT inhibitor prevents tumor formation in STING-/- KL xenografts, although they were not able to extend these data in terms of sustained STING activation, because available STING agonists do not penetrate into tumor cells.

Finally, given the mitochondrial dysfunction of KL cancer cells, Kitajima and colleagues investigated the contribution of nuclear and mitochondrial DNA (mtDNA) to the pool of cytosolic dsDNA. They found that cytosolic leakage of mtDNA, more than nuclear dsDNA, is essential to induce STING pathway activation by the DNA sensor cGAS. Top, percentage of TCGA patients with lung adenocarcinoma harboring mutations in KRAS and STK11 (KL, KRAS and TP53, and others. STK11 mutation is observed in 14% of lung adenocarcinomas, of which 7% co-occur with a KRAS mutation. Bottom, frequency of STK11 and TP53 mutations in patients with KRAS-mutant and wild-type (WT) lung adenocarcinoma. Significantly more STK11 mutations are seen in the KRAS-mutant population (37%) than in the KRAS WT population (10%; P < 0.001).
Taken together, this work provides strong evidence to support STAT1 as the main mediator of STING-dependent cytotoxicity and PD-L1 expression in lung adenocarcinomas with KRAS/STK11 (LKB1) co-mutation. This discovery has key therapeutic implications for patients with KL lung adenocarcinoma, an aggressive lung cancer subtype that is refractory to existing standard-of-care immune-checkpoint inhibitors. Indeed, in KL tumors, where STING is inactivated by LKB1, all downstream signals of antitumor immunity, including IRF3 and STAT1, are silenced and other protumorigenic signals (mainly mediated by NF-KB) sustain tumor progression. In summary, Kitajima and colleagues demonstrate for the first time that LKB1 is a key regulator of STING transcription, thus revealing a novel mechanism of immune resistance of KL lung adenocarcinoma.

The ability to convert a KL immune-resistant, noninflamed tumor into an immune-sensitive, inflamed tumor with LKB1 reconstitution or the use of STING agonists is supported by the in vitro model data. However, this is likely to prove more difficult in patients. Readily available therapeutic options that may increase the release of cytosolic DNA and STING activation include DNA-damaging therapies such as radiotherapy, chemotherapy, or DNA damage response (DDR) inhibitors (e.g., PARP inhibitors; ref. 7). However, direct stimulation of innate immunity through STING agonists is being actively pursued both in vivo and in clinical trials. For example, synthetic cyclic dinucleotides that mimic the STING ligand cGAMP are being explored as an approach to activate STING. One of these is MK-1454, which binds directly to STING in the cytosol. Preliminary data from the phase I trial of MK-1454 with pembrolizumab (NCT03010176) showed a safe tolerability profile and encouraging efficacy, with serum levels of STING-dependent cytokines increased posttreatment, confirming STING activation (8). However, MK-1454 requires intratumor injection, which poses obvious challenges in clinical practice (especially in patients without easily accessible tumors or with multifocal disease).

In November 2018, development of the first intravenous STING agonist (made up of two linked amidobenzimidazole compounds) with strong antitumor activity in a colon cancer model was reported by Ramanjulu and colleagues (9). Another strategy to target the STING pathway is by targeting the metabolism of nucleotides and stabilization of cGAMP (normally produced by cGAS in response to cytosolic DNA and then detected directly by STING). The oral agent MV-626 is a potent inhibitor of ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1; the phosphodiesterase of cGAMP) and has preclinical activity in combination with radiotherapy and anti–PD-L1 (10). Based on promising preclinical data, together with rapidly advancing options for STING targeting in the clinic, combinations of STING enhancers (e.g., STING agonists or ENPP1 inhibitors) with immunotherapy warrant further testing in KL lung adenocarcinomas. In addition, findings from Kitajima and colleagues suggest further investigation into the role of inhibitors of DNA methyltransferase or DDR, combined with STING enhancers or immunotherapy.

Finally, although this study focused on KRAS/STK11 comutated lung adenocarcinoma, it is likely that the findings have broader applications for overcoming immunotherapy resistance in other cancers with STK11/LKB1 mutation (Fig. 1D). For example, LKB1 loss is also found in lung cancers without KRAS mutations. Beyond lung cancer, STK11 sporadic mutations are found in cervical, breast, intestinal, testicular, pancreatic, and skin cancers; and germline STK11 mutations are associated with Peutz–Jeghers syndrome, a genetic disorder characterized by risk of various neoplasms, including intestinal and skin tumors. In conclusion, therapeutic strategies that can reactivate the LKB1 or STING pathway hold promise as a new way to boost anticancer immune response and to sensitize tumors that are resistant to anti–PD-1/PD-L1 agents in NSCLC and other cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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