A Genetic Mouse Model of Invasive Endometrial Cancer Driven by Concurrent Loss of Pten and Lkb1 Is Highly Responsive to mTOR Inhibition

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Abstract
Signals from the tumor suppressors PTEN and LKB1 converge on mTOR to negatively regulate its function in cancer cells. Notably, both of these suppressors are attenuated in a significant fraction of human endometrial tumors. In this study, we generated a genetic mouse model of endometrial cancer driven by concomitant loss of these suppressors to gain pathophysiological insight into this disease. Dual loss of Pten and Lkb1 in the endometrial epithelium led to rapid development of advanced endometrioid endometrial tumors with 100% penetrance and short host survival. The tumors displayed dysregulated phosphatidylinositol 3-kinase (PI3K) signaling pathway via hyperactivation of mTOR signaling. Treatment with a dual PI3K/mTOR inhibitor, BEZ235, extended the time before tumor onset and prolonged overall survival. The PI3K inhibitor GDC-0941 used as a single agent reduced the growth rate of primary tumor implants in Pten/Lkb1-deficient mice, and the mTOR inhibitor RAD001 was unexpectedly as effective as BEZ235 in triggering tumor regression. In parallel, we also found that ectopic expression of LKB1 in PTEN/LKB1-deficient human endometrial cancer cells increased their sensitivity to PI3K inhibition. Together, our results demonstrated that Pten/Lkb1-deficient endometrial tumors rely strongly on deregulated mTOR signaling, and they provided evidence that LKB1 status may modulate the response of PTEN-deficient tumors to PI3K or mTOR inhibitors. Cancer Res; 1–9. ©2013 AACR.

Introduction
Endometrial cancer is the most common gynecological malignancy in the United States, with more than 40,000 new cases diagnosed and ~7,000 deaths, annually (1). Aberrant activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway is frequently observed in a wide array of human cancers, including endometrial cancer (2–9). PI3Ks are central regulators of many essential cellular processes, including cell growth, proliferation, survival, and metabolism (2, 4, 10). Two common causes of aberrant activation of these regulators are activating mutations of PIK3CA (encoding the p110α catalytic subunit of PI3K) and loss-of-function mutations of the tumor suppressor PTEN: the consequence of either mutation is an accumulation of phosphatidylinositol (3, 4, 5). P3 at cell membranes, and subsequent constitutive activation of AKT, including the mTOR. Strikingly, recent comprehensive genomic characterization has identified activating mutations of PIK3CA and inactivating mutations or loss of PTEN in 53% and 67% of endometrial cancer (11) respectively, justifying the need to fully understand the importance of PI3K-signaling axis and AKT/mTOR activation in the pathogenesis of this malignancy. In addition to PTEN, the LKB1 tumor suppressor pathway also negatively regulates mTOR signaling. Germline defects of LKB1 result in Peutz–Jeghers Syndrome (PJS), a disorder that is characterized by intestinal hamartomas (12). Patients with PJS are at higher risks for epithelial cancers, including endometrial cancer (13), suggesting a tumor suppressive role of LKB1. LKB1 is a master upstream kinase of at least 13 downstream AMPK-related kinases (14): among these, AMPK is of central importance as a downstream effector of LKB1; AMPK suppresses the mTOR signaling pathway via phosphorylation of the tuberous sclerosis complex components 1 (TSC1) and 2 (TSC2; ref. 15). Loss of LKB1 protein expression is reported for 21% of primary endometrial tumors, and is correlated with activation of the...
mTOR pathway (16). Unlike PTEN whose loss can be caused by mutation, promoter methylation, and protein degradation (3, 17–19), mutation and homozygous deletion of LKB1 is a relatively rare event in endometrial cancer (3, 20) and the mechanism underlying decreased LKB1 protein level is unclear.

Genetic studies of mouse models have been critical for gaining insight into the role of specific genetic alterations in endometrial tumorigenesis (21). In one such model, biallelic deletion of Pten in mouse uterus was achieved by crossing Pten floxed mice with mice that express Cre recombinase under the control of the progesterone receptor promoter (PR cre/+; ref. 22); female offspring (as young as 1 month of age) from these breedings developed invasive endometrial cancer (22). However, because PTEN inactivation driven by PR-cre occurs during early embryogenesis (as early as embryonic day 10), and because progesterone receptor is also expressed in the stroma, this mouse model failed to faithfully mimic sporadic endometrial cancer in humans. In an alternative approach, somatic deletion of individual genes in the endometrium was achieved by delivering adenovirus-expressing Cre into the uterine lumen (23). With use of this method, recent studies in genetic mouse models have shown that somatic deletion of either Pten or Lkb1 specifically in the endometrium induces endometrial tumorigenesis with partial penetrance (20, 24). However, the effect of somatic loss of both tumor suppressors on the development of endometrial tumors has not been studied.

Intense efforts have been focused on developing inhibitors that target PI3K, AKT, and mTOR, which are major nodes in the PI3K pathway, and several such inhibitors are currently being evaluated in clinical trials for treating a variety of human cancers (2, 4, 5, 25), including endometrial cancer. Hence understanding the extent to which individual nodes involved in the PI3K pathway participate in tumorigenesis, and the mechanisms responsible for the aberrant activation of the PI3K pathway, is important for designing effective therapeutics for patients suffering from endometrial cancer (www.clinicaltrials.gov). PTEN and LKB1 are known to converge and suppress mTOR signaling; therefore, we hypothesize that loss of both suppressors in endometrium leads to hyperactivation of their downstream signaling and manifests as a worse disease phenotype. Here we test this hypothesis by evaluating the consequence of somatic concurrent loss of Pten and Lkb1 in mouse endometrium, and by assessing the effectiveness of PI3K pathway inhibitors in this mouse model.

Materials and Methods

Experimental mice and Ade-cre administration

Ptenlox/lox mice (Dr. Hong Wu, UCLA, Los Angeles, CA) and Lkb1lox/lox mice (NCI Mouse Repository) were fully backcrossed to C57BL/6 by 129Sv background. To generate compound Lkb1lox/lox mice (NCI Mouse Repository) were fully backcrossed to C57BL/6 by 129Sv background. To generate compound Lkb1lox/lox mice (NCI Mouse Repository) were fully backcrossed to C57BL/6 by 129Sv background. To generate compound Lkb1lox/lox mice (NCI Mouse Repository) were fully backcrossed to C57BL/6 by 129Sv background.

Materials and Methods

Tumor grafting and in vivo treatment studies

Endometrial tumors were isolated from Ptenlox/lox Lkb1lox/lox mice 10 weeks after Ade-Cre injection. The minced tumor tissue was grafted into the flank region of nude mice (NcrNu females, 8-week-old, Taconic) subcutaneously. The mice were given the dosages of the inhibitors as indicated in the figure legends by oral gavage. BEZ235 and RAD001 were obtained from Novartis Institutes for Biomedical Research (Basel, Switzerland), whereas GDC-0941 was purchased from MedChemexpress. GDC-0941 was dissolved in 0.5% methylcellulose/0.2% Tween 80, BEZ235 was dissolved in 10% NMP/90% PEG300 and RAD001 was formulated at 2% (w/v) in a microemulsion vehicle (Novartis). Tumor volumes were measured twice a week with calipers and calculated according to the following formula: tumor volume = (length × width²)/2.

Cell cultures and plasmids

ETN-1, HEC108, and other human endometrial cancer cell lines used in this study were kindly provided by Dr. Gordon B. Mills (MD Anderson Cancer Center, Houston, TX; ref. 3). pBABE-puro and pBABE-Flag-LKB1 plasmids were kindly provided by Dr. Lewis C. Cantley (26).

Cell proliferation assay

Cells were plated in 96-well microtiter plates at density of 5,000 cells per well. After 24 hours, cells were treated with serial dilutions of GDC-0941. Cell proliferation was assessed after 72 hours of treatment by the WST-1 assay (Roche).

Western blot analysis

Tumors were homogenized in ice-cold RIPA buffer (Sigma) containing protease inhibitor cocktail (Roche) and phosphatase inhibitor (Roche). For tumor and cell lysates, standard Western blotting was conducted as described (37) using antibodies against p-AKT (Ser473), p-AKT(Thr308), p-S6RP (Ser235/Thr236), S6RP, p-4EBP1 (Ser65), 4EBP1 and PTEN (Cell Signaling), LKB1 (Santa Cruz), and CK8 (Troma-1, University of Iowa).
rabbit IgG (Molecular Probes) were used to visualize Western blots on an Odyssey scanner (Li-Cor).

**Reverse phase protein array analysis**
Reverse phase protein array (RPPA) analysis and the patient dataset are described in The Cancer Genome Atlas (https://tcga-data.nci.nih.gov/tcga/).

**Statistical analyses**
The survival of mice was analyzed by Kaplan–Meier log-rank test. Quantitative results were analyzed by 2-tailed unpaired Student t test. \( P < 0.05 \) was considered statistically significant.

**Results**

**Concurrent low abundance of PTEN and LKB1 found in a fraction of human endometrial cancers**
Previous studies have reported frequent alterations of PTEN in endometrial cancer, and shown association of decreased LKB1 protein with advanced disease stage (3, 16–20). However, it was unclear whether concurrent loss of PTEN and LKB1 exists in endometrial cancer. Here we evaluated the abundance of PTEN and LKB1 proteins by RPPA analysis on a large cohort of primary endometrial tumors (Fig. 1A). The abundance of each protein, PTEN or LKB1, was defined as high, intermediate, or low. Low abundance of PTEN and LKB1 was found in 35% (56/159) and 28% (44/159) of endometrioid cancers, respectively, consistent with the previous findings that loss of Pten and Lkb1 are both frequent events in patients with endometrial cancer (3, 9, 16, 20, 27). Although the observed frequency of coexisting low abundance of PTEN and LKB1 (6.3%) is lower than the expected frequency (8.8%) in grade 1 and grade 2 tumors, the observed frequency of coexistence (14.9%) is higher than the expected frequency (12.0%) in grade 3 tumors, suggesting there is an enrichment of concurrent low abundance of PTEN and LKB1 in high-grade tumors. This finding prompted us to investigate the potential cooperative effect of these 2 events on the pathogenesis of endometrial cancer.

![Figure 1. Concurrent loss of Pten and Lkb1 in mouse endometrium led to the development of invasive endometrial tumors. A, RPPA analysis of PTEN and LKB1 proteins in a cohort of primary human endometrioid endometrial tumors (The Cancer Genome Atlas, https://tcga-data.nci.nih.gov/tcga/). The abundance of each protein, PTEN or LKB1, was defined as high, intermediate, or low. B, Kaplan–Meier survival curves for mice of indicated genotypes as a function of days after Ade-cre administration. \( P \text{ten}^{lox/lox} \text{Lkb1}^{lox/lox} (n = 8), \text{Lkb1}^{lox/lox} (n = 9), \text{and Pten}^{lox/lox} \text{Lkb1}^{lox/lox} \) females (n = 23). \( P < 0.0001 \) (log-rank test); mean latency, 127 days. C, injection of Ade-Cre into the right uterine horn of \( Pten^{lox/lox} \text{Lkb1}^{lox/lox} \) female mice resulted in hemorrhagic ascites (left) and a large uterine tumor (right, arrow); arrowhead, normal uterine horn. D, histopathology of normal uterus (top) and Pten Lkb1-deficient endometrial carcinomas (bottom). Scale bars, 100 \( \mu \text{mol/L} \) (top left) and 500 \( \mu \text{mol/L} \) (bottom left); 50 \( \mu \text{mol/L} \) (middle); 25 \( \mu \text{mol/L} \) (right).
Concurrent deletion of Pten and Lkb1 in endometrium resulted in rapid development of aggressive endometrial tumors

Pten and Lkb1 have been revealed as potent tumor suppressors of endometrial cancer in experimental mouse models (16, 20, 22–24, 28), both of which also converge on and negatively regulate mTOR signaling. To determine whether deregulation of these 2 pathways may have a cooperative impact on endometrial carcinogenesis, we used a previously described in vivo approach (23) to conditionally delete targeted genes in the endometrial epithelium of mice homozygous for floxed alleles of Pten and/or Lkb1 (Pten\textsuperscript{loxP/loxP} and/or Lkb1\textsuperscript{loxP/loxP}). Cohorts of Pten\textsuperscript{loxP/loxP} (n = 6), Lkb1\textsuperscript{loxP/loxP} (n = 8), or Pten\textsuperscript{loxP/loxP} Lkb1\textsuperscript{loxP/loxP} (n = 23) mice were administered with adenovirus expressing Cre (Ade-Cre) into the lumen of the right uterine horn at 8 to 12 weeks of age. Between 8 to 28 weeks after Ade-Cre injection, Pten\textsuperscript{loxP/loxP} Lkb1\textsuperscript{loxP/loxP} mice presented with overt disease state, including palpable tumor mass, abdominal bloating, and decreased mobility. These mice developed tumors in the uterine horn injected with Ade-cre but not in the contralateral, uninjected uterine horn, with 100% penetrance and a median survival of 127 days after Ade-Cre injection (Fig. 1B). Substantial hemorrhagic ascites in the peritoneal cavity were found in 69.5% (16/23) of the mice at necropsy (Fig. 1C). Strikingly, in 52.2% (12/23) cases, the uterine cancer cells even broke away from the uterus and invaded adjacent organs such as liver and peritoneal wall (Supplementary Fig. S1). Cre-mediated recombination resulted in null alleles of Pten and Lkb1 (Supplementary Fig. S2). Consistently, both Pten and Lkb1 proteins were found lost in epithelial tumor cells but remained evident in the surrounding stromal cells (Supplementary Fig. S3). In contrast, no apparent phenotype or mortality was observed from either Pten\textsuperscript{loxP/loxP} or Lkb1\textsuperscript{loxP/loxP} mice following administration of Ade-Cre for up to 10 months (Supplementary Table S1). Subsequent histopathological examination identified hyperplastic lesions in Pten-deficient uterus but well-differentiated endometrial cancer lesions in Lkb1-deficient uterus with partial penetrance (Supplementary Figs. S5 and S6 and Table S1), the latter of which is consistent with a previous report (20). Together, the complete penetrance and short latency of tumorogenesis seen in this mouse model suggest that cooperating genetic abnormalities in both Pten and Lkb1 are sufficient to form fully malignant endometrial tumors.

Histologically, the uterine tumors developed in Pten\textsuperscript{loxP/loxP} Lkb1\textsuperscript{loxP/loxP} were aggressive endometrioid endometrial adenocarcinoma. The endometrial layer has lost well-differentiated glandular structures as those seen in normal uterus and the chaotically growing endometrial epithelial cells were often moderately to poorly differentiated in nature and tightly intermingled with collagen-rich stroma (Fig. 1D and Supplementary Fig. S7). In all cases, endometrial cells were seen to completely invade through the entire myometrial wall and reach the serosal surface of the uterus (Fig. 1D). Histological analyses also identified macroscopic metastases with endometrioid glandular morphology in the lung in 65.2% (15/23) of cases (Supplementary Fig. S8), strongly supportive of a high metastatic potential of this tumor model. Of note, in some areas of the endometrial tumors, we also noticed the stroma adjacent to the invasive adenocarcinoma exhibits a desmoplastic response resembling those seen in human cancer (Supplementary Fig. S9). Together, the tumor driven by combined endometrial-specific loss of Pten and Lkb1 represents a mouse model of highly invasive endometrial cancer that recapitulates the advanced stage of the human disease and thus provides a useful platform for preclinical testing of molecularly targeted therapeutics in treating human high-grade endometrioid endometrial cancer.

Dysregulated signaling pathways in endometrial tumors driven by concurrent loss of Pten and Lkb1

To assess signaling alterations in Pten Lkb1-deficient endometrial tumors, we analyzed the in vivo signaling cascades known to be downstream of Pten and Lkb1. Lkb1 is known to directly phosphorylate and activate Ampk to suppress mTOR pathway (15). Consistent with this, the normal endometrial glands revealed strong phosphorylation of Ampk (Thr172; Supplementary Fig. S10). In contrast, phosphorylation of AMPK and ACC is almost completely abolished in endometrial tumors, suggesting that these tumors have lost the Lkb1-Ampk signaling axis that controls mTOR activity. Akt, a serine-threonine kinase, is known to be aberrantly phosphorylated and activated in response to PI3K activation as a result of Pten loss (10). In line with this, we found significantly increased levels of p-AKT in endometrial tumors (Supplementary Fig. S10). mTOR and S6RP, a downstream effector of mTOR, which are downstream of both AKT and AMPK, are strongly phosphorylated, consistent with aberrant activation of mTOR signaling because of concomitant loss of Pten and Lkb1. The dysregulated Lkb1/Ampk signaling and hyperactivated AKT/mTOR signaling seen in the endometrial tumors raises the possibility that these tumors might be dependent on PI3K-AKT and mTOR signaling pathways for maintenance of the malignant phenotype and thus hypersensitive to inhibition of particular nodes in the signaling pathway and in particular those downstream of the TSC1/2 convergence point for both pathways.

Dual inhibition of PI3K and mTOR effectively treated Pten Lkb1-deficient endometrial tumors

We next evaluated the effect of NVP-BEZ235, a potent dual kinase inhibitor that targets both PI3K and mTOR. We administered Ade-cre to the uterine lumen of a cohort of Pten\textsuperscript{loxP/loxP} Lkb1\textsuperscript{loxP/loxP} mice. At 5 weeks after Ade-cre administration, drug treatment by BEZ235 (35 mg/kg/day) or vehicle was initiated. Strikingly, 6 weeks of BEZ235 treatment greatly decreased disease progression, as evidenced by a significant decrease in uterine weight of mice in the drug-treated group compared with those in the vehicle-treated group (Fig. 2A and B, panels a and b, P < 0.0005). By the end of 3 months’ drug treatment, all remaining mice in the vehicle-treated group succumbed to death whereas all remaining BEZ235-treated mice survived (Fig. 2C, P < 0.0005). Thus, BEZ235 significantly prolonged survival, representing a promising.
molecular-targeted therapeutics in treating endometrial cancer driven by concurrent loss of Pten and Lkb1. Furthermore, immunohistochemical analysis revealed that there was a marked decrease in the abundance of both p-AKT and p-S6RP in the endometrial cancers treated with BEZ235 (Fig. 2D), consistent with inhibition of both PI3K and mTOR by this drug. We also found that the cell proliferation as measured by Ki67 staining significantly decreased in endometrial tumors treated with BEZ235 (Fig. 2E). Conversely, the apoptotic cell death as assessed by cleaved caspase-3 markedly increased in BEZ235-treated tumors (Fig. 2E). Therefore, decreased cellular proliferation and increased apoptosis are, at least in part, responsible for the inhibited tumor growth in mice treated with BEZ235.

Differential responses of Pten Lkb1-deficient endometrial tumors to inhibitors targeting PI3K and/or mTOR

Loss of Pten function confers cancer cells increased sensitivity to PI3K or mTOR inhibition, each as a single agent (29–32). Meanwhile, it has been recently shown that Lkb1 is critical in suppressing mTOR-mediated endometrial cancer (20, 28). We next determined if endometrial tumors driven by combined inactivation of both Pten and Lkb1 are sensitive to selective inhibitors of mTOR and PI3K as single agents. To facilitate the comparison of treatment responses, we first established subcutaneously transplanted endometrial tumors in immunodeficient mice and then subjected tumor-bearing mice with similar sized tumors to drug treatment. Consistent
with the genetic mouse model, NVP-BEZ235 (35 mg/kg/day) treatment resulted in dramatic tumor regressions (Fig. 3A). Surprisingly, mTOR inhibition by RAD001 (7.5 mg/kg/day) also caused significant and persistent tumor regressions, a strong inhibitory effect comparable to that produced by BEZ235 treatment (Fig. 3A). In contrast, a high dose (125 mg/kg/day) of the PI3K selective inhibitor GDC-0941 was only able to slow down the tumor growth, indicating that targeting PI3K activity alone was not sufficient to shrink these tumors. To examine PI3K/mTOR signaling changes in response to different treatment modalities, we isolated tumors from drug-treated mice for 3 days and analyzed tumor lysates by Western blot analysis. Although GDC-0941 abrogated the phosphorylation of Akt, it had little effect on mTOR activity as assessed by phospho-S6RP levels (Fig. 3B). In contrast, both RAD001 and BEZ235 substantially diminished mTOR signaling (Fig. 3B). In line with previous reports (33, 34), we also found that mTOR inhibition by RAD001 resulted in increased AKT phosphorylation, albeit modest, as well as upregulation of ERK signaling. Despite of these signaling alterations, Pten Lkb1-deficient endometrial tumors regressed in response to mTOR inhibition (Fig. 3A). Together, these results suggest that endometrial tumors driven by concurrent loss of Pten and Lkb1 are highly dependent on hyperactivated mTOR signaling, suggesting that mTOR-directed therapy is an effective treatment strategy for this type of endometrial cancer.

**Ectopic expression of LKB1 sensitizes PTEN LKB1-deficient endometrial cancer cells to PI3K inhibition**

Our finding that targeting PI3K is not sufficient to inhibit mTOR activity or block the growth of Pten-deficient endometrial tumors lacking Lkb1 suggests that loss of Lkb1 may contribute to the reduced sensitivity of endometrial cancer cells to PI3K inhibitors. To this end, we have analyzed a panel of 12 human endometrial cancer cell lines and found that at least 2 of them, HEC108 and ETN-1, are deficient of both PTEN and LKB1 (Fig. 4A). Because mTOR signaling is a convergent node of both PI3K- Akt and LKB1-AMPK signaling axes and Pten Lkb1-deficient endometrial tumors are highly dependent on mTOR activation in our genetic mouse model, we next determined whether add-back of LKB1 in PTEN LKB1-deficient endometrial cancer cells affects their response to PI3K inhibition. Although GDC-0941 treatment reduced p-AKT in both ETN-1 and HEC108 cells regardless of LKB1 expression levels, it had little effect on mTOR activity in vector-expressing cells (Fig. 4B). In contrast, GDC-0941 significantly reduced phosphorylation of both S6RP and 4EBP1, downstream effectors of mTOR signaling, in the corresponding cells expressing LKB1 (Fig. 4B and C). Furthermore, we show that, compared with their respective vector-expressing cells, LKB1-expressing ETN-1 and HEC108 cells exhibited a significantly reduced proliferation in response to PI3K inhibition by GDC-0941 (Fig. 4D). Together, these results suggested that the status of LKB1 may alter signaling response to PI3K inhibition in PI3K-deficient endometrial cancer cells and modulate their sensitivity to PI3K and mTOR inhibitors.

**Discussion**

In this study, we developed a genetic mouse model of advanced endometrial cancer that recapitulates a subset of human endometrial concurrent low abundance of Pten and Lkb1 proteins. Concomitant loss of Pten and Lkb1 in mouse endometrial epithelium leads to rapid development of high-grade endometrioid endometrial adenocarcinomas with 100% tumor penetrance and a short survival. Consistent with the previous finding by Contreras and colleagues (20), we also found that somatic biallelic deletion of Lkb1 in mouse endometrium led to the development of invasive but well-differentiated endometrioid endometrial adenocarcinoma with partial penetrance (Supplementary Fig. S6 and Table S1). Although somatic biallelic ablation of Pten in mouse endometrium only induces hyperplastic lesions by 10 months in our study (Supplementary Fig. S5 and Table S1), a previous study using the same genetic approach reported invasive endometrial adenocarcinoma with partial penetrance by 8 months (24). This discrepancy may be because of differences in the genetic background of the mice and/or the titer or infection efficiency of the adenovirus used in these 2 studies. As the disease phenotypes seen in mice with simultaneous loss of Pten and Lkb1 are much more severe
than those in mice with loss of either gene alone in our study (Fig. 1 and Supplementary Table S1), our data reveal that Pten and Lkb1 have a cooperative effect in suppression of endometrial tumorigenesis, consistent with clinical data that concurrent loss of these tumor suppressors is enriched in high grade of endometrial cancer.

A surprising and potentially meaningful finding of this study is that inhibition of mTOR activity by RAD001 as a single agent was as effective as the dual PI3K/mTOR inhibitor NVP-BEZ235 in treating advanced endometrial tumors driven by loss of both Pten and Lkb1, suggesting a critical role of mTOR signaling in this type of cancer. This is intriguing as inhibition of mTOR can lead to increased activity of PI3K/AKT as well as increased MAPK activity (33, 34), which has been suggested to compromise drug efficacy in the treatment of other cancer types. However, our finding that mTOR inhibition is effective in treating advanced endometrial tumors is concordant with the results from recent clinical trials testing mTOR inhibitors in endometrial cancer. These studies have reproducibly reported stable disease in a significant number of cases, even for patients with metastatic or recurrent endometrial cancers (35, 36).

Although mTOR inhibitors have showed encouraging results in endometrial cancers, it is worth noting that clinical trials of mTOR inhibitors had been initiated long before PI3K inhibitors became available for clinical development. Moreover, given the fact that genetic alterations in the components of the PI3K pathway, for example PI3K, PTEN, and PIK3R1 genes, are common in endometrial cancers (53%, 65%, and 33%, respectively; ref. 11), it has been postulated that these cancers might have acquired dependence on PI3K activation and thus are likely to show better response to new agents targeting above mTOR. Indeed, a significant number of clinical trials testing PI3K and AKT inhibitors as single agents are currently underway for the treatment of endometrial cancers (www.clinicaltrial.gov).

Although clinical trial results are awaited to inform whether PI3K inhibitors have superior efficacy in tumors harboring PI3K pathway alterations, there is a crucial need for preclinical studies to identify additional molecular driver events that modulate tumor responses to PI3K-targeted cancer therapy. For the first time, our work using a well-defined genetic mouse model has revealed that endometrial tumors harboring PI3K pathway activation with a perturbed LKB1/AMPK signaling
axis are actually more sensitive to an mTOR inhibitor than a PI3K inhibitor as single agents. Notably, our data are also in concordant with a previous work in non–small cell lung cancer, in which Lkb1 loss was found associated with greater resistance to PI3K/AKT inhibitor and MEK inhibitors as single agents (37). Furthermore, using human endometrial cancer cells that are deficient in PTEN and LKB1, we show that re-expression of LKB1 in these PTEN-deficient endometrial cancer cells rendered them more susceptible to growth inhibition by PI3K inhibitors. Thus, LKB1 may act as a "molecular switch" that modulates the dependency of PTEN-deficient tumor cells on the PI3K/AKT or mTOR signaling. Moreover, our study also provides potential translational insights that the status of LKB1 and its signaling axis may be a useful predictive marker in clinical trials of selecting appropriate modalities inhibiting the PI3K-AKT-mTOR signaling pathway.

Disclosure of Potential Conflicts of Interest

A. Myers is employed as a clinical program leader in Novartis. L.C. Cantley is a consultant/advisory board member of Novartis. G.B. Mills has a commercial research grant from AstraZeneca, PTV Ventures, and Spindle Top Ventures. G.B. Mills is also a consultant/advisory board member of AstraZeneca, Bind, Critical Outcome Technologies, Hanbio Korea, Nuevolution, and Symphogen. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Cheng, R.T. Bronson, J.J. Zhao
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