Loss of Lkb1 Provokes Highly Invasive Endometrial Adenocarcinomas

Cristina M. Contreras,1,2 Sushma Gurumurthy,5 J. Marshall Haynie,1,2 Lane J. Shirley,1,2 Esra A. Akbay,1,2 Shana N. Wingo,1 John O. Schorge,2,3,4 Russell R. Broaddus,7 Kwok-Kin Wong,6 Nabeel Bardeesy,5 and Diego H. Castrillon1,2

1Department of Pathology, 1Simmons Comprehensive Cancer Center, 1Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, and 3Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, Texas; 1Department of Medicine, Massachusetts General Hospital Cancer Center and Harvard Medical School; 1Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts; and 1Department of Pathology, M. D. Anderson Cancer Center, Houston, Texas

Abstract
Mutations in the LKB1 tumor suppressor gene result in the Peutz-Jeghers syndrome, an autosomal dominant condition characterized by hamartomatous polyps of the gastrointestinal tract (1, 2). Peutz-Jeghers syndrome is also associated with a dramatically increased risk of epithelial malignancies at other sites, including the female reproductive tract. Here we show that female mice heterozygous for a null Lkb1 allele spontaneously develop highly invasive endometrial adenocarcinomas. To prove that these lesions were indeed due to Lkb1 inactivation, we introduced an adenoaviral Cre vector into the uterine lumen of mice harboring a conditional allele of Lkb1. This endometrial-specific deletion of the Lkb1 gene provoked highly invasive and sometimes metastatic endometrial adenocarcinomas closely resembling those observed in Lkb1 heterozygotes. Tumors were extremely well differentiated and histopathologically distinctive and exhibited alterations in AMP-dependent kinase signaling. Although Lkb1 has been implicated in the establishment of cell polarity, and loss of polarity defines most endometrial cancers, Lkb1-driven endometrial cancers paradoxically exhibit (given their highly invasive phenotype) normal cell polarity and apical differentiation. In human endometrial cancers, Lkb1 expression was inversely correlated with tumor grade and stage, arguing that Lkb1 inactivation or down-regulation also contributes to endometrial cancer progression in women. This study shows that Lkb1 plays an important role in the malignant transformation of endometrium and that Lkb1 loss promotes a highly invasive phenotype. [Cancer Res 2008;68(3):759–66]

Introduction
Inherited germ line mutations in the LKB1 gene result in the Peutz-Jeghers syndrome, an autosomal dominant condition characterized by hamartomatous polyps of the gastrointestinal tract (1, 2). Peutz-Jeghers syndrome is also associated with a significantly elevated (>15-fold) general cancer risk, suggesting that LKB1 plays a broad tumor suppressor role (3). Consistent with this idea, somatic inactivation of the LKB1 gene by point mutation (or other mechanisms such as promoter hypermethylation) has more recently been shown to be a common event driving carcinomas of the gall bladder, pancreas, and lung (4–7).

Peutz-Jeghers syndrome is strongly associated with clinically and histopathologically distinctive neoplasms of the female reproductive tract, including the ovary and uterine cervix. In the endocervix, Peutz-Jeghers syndrome is associated with an extremely well-differentiated variant of endocervical adenocarcinoma (minimal deviation adenocarcinoma of the endocervix, also known as adenoma malignum). The histopathologic diagnosis of this tumor (i.e., in biopsies) can be difficult because the malignant glands so closely resemble normal endocervical glands. Their benign histologic appearance is deceptive, however, because these tumors are invasive, often large, and clinically aggressive (8).

The Lkb1 protein participates in at least two distinct but potentially overlapping biological pathways that seem to synergize in tumorigenesis. In the mouse, Lkb1 establishes cell polarity in cortical neurons (9) and colonic epithelium (2), and it has been suggested that most of the consequences of Lkb1 deficiency in tumors are indirect consequences of cell polarization defects (10). Loss of cell polarity strongly correlates with aggressive and invasive malignant cell growth (11) and is a general feature of adenocarcinomas, including those of endometrial origin (12). Second, Lkb1 directly phosphorylates and regulates AMP-dependent kinase (AMPK), a sensor of intracellular ATP levels. AMPK controls protein synthesis by regulating the mammalian target of rapamycin (mTOR) through phosphorylation of TSC2 (tuberin; ref. 13). Through this pathway, loss of Lkb1 activity in tumors may derepress protein synthesis, thereby promoting cell growth, proliferation, and tumorigenesis. Intriguingly, the regulation of cell polarity by Lkb1 seems to be at least partially mediated by AMPK, which, in addition to its roles in the mTOR pathway, also regulates the assembly of epithelial tight junctions and thus directly contributes to the maintenance of epithelial cell polarity (14–16). Although these findings suggest that AMPK is of central importance as a downstream effector of Lkb1 inactivation, Lkb1 is also the master upstream kinase of at least 13 AMPK-related kinases (17), suggesting that Lkb1 may contribute to tumorigenesis through other as yet unknown mechanisms.

A mouse Lkb1 knockout model of Peutz-Jeghers syndrome closely mimics the human condition. Mice heterozygous for a null Lkb1 mutation are viable and externally normal, but develop intestinal polyps identical to those seen in Peutz-Jeghers syndrome patients (Lkb1+/− embryos die early in gestation; ref. 18). Here we report that Lkb1−/− mice also spontaneously develop recurring distinctive neoplasms within the uterine corpus. These tumors are extremely well differentiated yet highly invasive, similar to the uterine endocervical neoplasms in women with Peutz-Jeghers syndrome, a notable finding given that endocervical and endometrial epithelia share a common embryologic origin as Mullerian duct derivatives and are directly contiguous within the uterus. Inactivation of a
floxed \( Lkb1 \) allele at 6 weeks of age proved that these endometrial cancers were the result of \( Lkb1 \) inactivation and arise secondarily following normal uterine development. Further analyses showed that tumor cells with biallelic \( Lkb1 \) inactivation do not have gross defects in cell polarity, suggesting that \( Lkb1 \) restrains endometrial tumor cell growth and invasion via biological pathways separable from the establishment of cell polarity per se. Lastly, we show that low \( Lkb1 \) expression in human endometrial cancers strongly correlates with invasiveness. Our results thus show that \( Lkb1 \) loss may have clinical utility as a prognostic marker.

Materials and Methods

Colony generation and Ad-Cre administration. This study was approved by an institutional animal care and use committee. The mouse null (–) and floxed (\( L \)) alleles of \( Lkb1 \) used in this study have previously been reported. Both were backcrossed for six generations to strain FVB, and genotyping was done as described (18). Experimental \( Lkb1^{+/–} \) animals were generated by outcrossing to FVB mice; \( Lkb1^{+/-} \) mice were propagated as homozygotes. Intrauterine Ad-Cre injections of \( Lkb1^{+/-} \) mice were done as described (19) with modifications: Ad5CMVCre adenovirus (University of Iowa) was stored in 3% sucrose/PBS at 6 \( \times \) 10\(^{11}\) plaque-forming units (pfu)/\( \mu l \) in 20-\( \mu l \) aliquots, thawed immediately on ice before use, diluted in MEM (Sigma) to a final concentration of 2.4 \( \times \) 10\(^{9}\) pfu/\( \mu l \), and incubated at room temperature \( \times \) 30 min. Female mice (6–8 weeks old) at diestrus or postestrus were anesthetized, and Genomate 57-mm multiFlex Tips (ISCBioExpress) connected to 1-\( ml \) syringes were used to deliver 50 \( \mu l \) of adenovirus/MEM through the cervical os and into each uterine horn.

Tissue microarray generation and analysis, histologic techniques, immunohistochemistry, and RNA in situ hybridization. The tissue microarray contained 2 \( \times \) 0.4-mm cores of paraffin blocks from each of 190 randomly selected primary endometrial cancers representing a broad range of histologic subtypes. Grade and stage were determined at the time of surgery. Grade 1 tumors were classified as low grade and grade 2 + 3 tumors as high. For immunohistochemistry, 5-\( \mu m \) sections were deparaffinized and hydrated in an ethanol series. Slides for all antibodies except lectin were subjected to antigen retrieval by boiling in 10 mmol/L Na citrate and cooled at room temperature \( \times 20 \) min; slides for lectin immunohistochemistry were not subjected to antigen retrieval. Antibodies used were lectin, Ulex Europaeus (Sigma) at 1:100 dilution, smooth muscle actin 1:1000 (Sigma), phosphorylated (p-) eukaryotic translation initiation factor 4E –binding protein 1 (4EBP1; Thr47) (Cell Signaling Technologies), p-Akt(Ser473) (Cell Signaling Technologies), p-AMPK (Cell Signaling Technologies), phosphorylated acetylcoenzymeA carboxylase (Cell Signaling Technologies), phosphorylated S6 kinase (p-S6K; Cell Signaling Technologies), and Lkb1 1:100 (18). The detection system was Immpress (Vector). For RNA in situ hybridization, sense and antisense probes of a full-length \( Lkb1 \) cDNA were made using the DIG RNA Labeling Mix (Roche) and in situ hybridization was done according to standard protocols (20). For apoptotic and mitotic counts in primary tumors, apoptotic bodies and mitotic figures were counted per stan-
dard morphologic criteria on routine H&E sections. Counts were obtained from normal endometrial and tumor epithelium from the same animal.

Western blot analysis. Normal and tumorous uterine tissues were homogenized in lysis buffer [50 mmol/L HEPES, 250 mmol/L NaCl, 2 mmol/L EDTA, 25% Glycerol, 1% NP40, 0.1% SDS, protease inhibitors (Roche), and phosphatase inhibitors (Calbiochem)] on ice. Retroviruses expressing Lkb1 or Lkb-KD were generated by transfecting 293T cells with pBabe-Lkb1 or Lkb-KD plasmids and collecting supernatant media at 36 h posttransfection. Lkb1-deficient squamous cell carcinoma cell lines were infected with retroviruses expressing Lkb1 or Lkb-KD, \(^8\) selected with puromycin and harvested in lysis buffer 48 h postinfection, and then subjected to SDS-PAGE and immunoblotted with indicated antibodies. Cell lines were derived from squamous cell carcinoma as described (21).

\(^8\) In preparation.
Electron microscopy. Tissues were cut into 1-mm cubes, fixed in 2% glutaraldehyde, 0.1 mol/L sodium cacodylate, and postfixed in 1% OsO₄/2% uranyl acetate. Samples were dehydrated, rinsed with propylene oxide, embedded in Epon, polymerized overnight at 60°C, sectioned (50–70 nm) using a Reichert ultracut E microtome, and mounted on formvar-carbon-coated copper grids. Images were obtained on a JEOL 1200EX transmission electron microscope.

Results

Female Lkb1⁻/⁻ mice spontaneously develop uterine tumors (endometrial adenocarcinomas). The well-documented association between Peutz-Jeghers syndrome and tumors of the female reproductive tract prompted an investigation of the female reproductive tract in Lkb1⁻/⁻ mice. Cohorts of Lkb1⁻/⁻ and sibling wild-type mice were generated and aged. As previously reported, by 43 weeks of age, 40 of 59 Lkb1⁻/⁻ mice (versus 0 of 65 wild-type control mice; \( P = 2.5 \times 10^{-18} \), Fisher’s exact test) developed gastrointestinal polyps resulting in symptoms of gastrointestinal obstruction. Of surviving females up to 55 weeks of age, 4 of 15 (26.7%) also had large uterine tumors readily detectable on routine gross examination. Increased tumor incidence was not observed in anatomic sites other than the uterus and gastrointestinal tract. Serial sectioning and microscopic analysis of uteri from all 15 females revealed the presence of smaller but histologically identical tumors in an additional 4 of 15 (26.7%) of females. Thus, in total, 8 of 15 (53%) of surviving Lkb1⁻/⁻ females spontaneously developed uterine neoplasms by 55 weeks of age (versus 0 of 54 wild-type control mice; \( P = 7.7 \times 10^{-7} \), Fisher’s exact test).

Histologic analysis revealed that these uterine neoplasms were histologically distinctive, well-differentiated endometrial adenocarcinomas. The uterus consists of three layers: endometrium (epithelium plus stroma), myometrium, and serosa (the outer single-cell layer of mesothelium); the normal endometrial-myometrial interface is shown by a dashed line in Fig. 1A. The Lkb1⁻/⁻ uterine tumors consisted of endometrial-type glands invading into myometrium (Fig. 1A), where endometrial glands are not normally present. Higher magnification revealed that the tumors consisted of very well-differentiated glands diffusely infiltrating through surrounding tissue. Despite their well-differentiated appearance, the tumors frequently invaded through the entire myometrial thickness and reached the serosa (Fig. 1B). To better define the uterine layers and confirm myometrial invasion in Lkb1⁻/⁻ mice, immunohistochemistry was done against smooth muscle actin, revealing that in tumor areas, myometrium was normally developed yet extensively invaded by well-differentiated glands of adenocarcinoma (Fig. 1C).

Furthermore, tumor glands were not associated with endometrial
stroma, arguing against adenomyosis (maldevelopment of normal endometrium within the myometrium) and consistent with true invasion (Fig. 1C, inset).

Northern blot analysis showed that Lkb1 is broadly expressed in many mouse tissues, including the uterus (data not shown). To further define the cellular compartments where Lkb1 is expressed within the uterus, RNA in situ hybridization was done. Lkb1 was detectable throughout the uterus but most highly expressed in the endometrial epithelium, consistent with a cell-autonomous role in the regulation of endometrial cell growth (Fig. 1D). No significant variation in Lkb1 expression was observed on different days of the estrus cycle, suggesting that Lkb1 expression is not steroid hormone dependent (data not shown).

**Postnatal endometrial-specific gene inactivation shows that Lkb1 is a bona fide endometrial tumor suppressor.** The above findings were most consistent with neoplastic growth but did not formally exclude a developmental abnormality such as adenomyosis. To exclude this possibility and to determine if Lkb1 functions in a cell-autonomous manner, we injected adenovirus expressing the Cre recombinase (Ad-Cre) into the uterine lumen of female mice homozygous for a floxed Lkb1 allele (L). As previously shown, this approach results in efficient and transient transduction of endometrial epithelium but, because of the presence of tight junctions, not of endometrial stromal or other cell types within the uterus (19).

Cohorts of 17 Lkb1<sup>L/L</sup> homozygous floxed and 30 control wild-type female mice were injected with Ad-Cre at 6 weeks of age and euthanized at 9 months posttreatment. PCR confirmed Cre-mediated recombination and the presence of the null allele in uterine DNA, but not in control tail DNA (data not shown). Of the 17 Lkb1<sup>L/L</sup> mice, 11 (65%) developed uterine tumors, versus 0 of 30 of controls mice similarly treated with Ad-Cre (P = 7.1 × 10<sup>−7</sup>, Fisher’s exact test). The majority of these tumors were confined to the uterus, but one was diffusely metastatic within the peritoneum (Fig. 2A). No extraterine tumors were observed. Histologically, the tumors in Ad-Cre–treated Lkb1<sup>L/L</sup> females were identical to those in Lkb1<sup>L/L</sup> females. The tumors in Lkb1<sup>L/L</sup> females similarly involved the myometrium (Fig. 2B) and consisted of diffusely infiltrating well-differentiated glandular tumor epithelium that frequently invaded through the entire thickness of the myometrium, often reaching the serosal surface. Distant metastatic tumor glands were indistinguishable from those in primary

**Figure 3.** Misregulation of AMPK signaling in endometrium following Lkb1 deletion. A, immunohistochemical studies. For p-S6K, most areas of tumor showed few positive cells similar to normal control endometrium (arrows), but small foci (comprising <20% of tumor epithelium) contained somewhat increased numbers of positive cells (inset). All images are of same magnification except the top right image, which is shown at 2.5× higher magnification to highlight abundant Lkb1 expression in stroma (S). Slides were immunostained, developed in diaminobenzidine, and counterstained with hematoxylin. B, Western blot analysis of another tumor also shows decreased levels of p-AMPK. Lkb1-deficient squamous carcinoma cells (SCC) with enforced expression of wild-type or kinase-dead (KD) forms of Lkb1 served as additional controls.
Loss of Lkb1 protein in endometrial tumor cells results in misregulation of AMPK signaling downstream of Lkb1. PCR analysis of DNA from various tissues in Ad-Cre–treated animals showed Cre-mediated recombination and the presence of the null allele in uterus but not other tissues (data not shown). Immunohistochemistry confirmed that Lkb1 protein was indeed undetectable in tumor epithelial cells, whereas it remained detectable in adjacent stromal cells (Fig. 3A). To gain insights into the molecular mechanisms by which Lkb1 loss contributes to endometrial carcinogenesis, we analyzed the activation/phosphorylation status of selected components of the kinase cascade downstream of Lkb1. These in situ studies permitted analysis of pathway status in Lkb1 endometrial tumors although they are composed predominantly of stromal cells.

Lkb1-mediated repression of mTOR signaling through the AMPK-Tsc pathway is thought to be important for tumor suppression (2). Endometrial tumor cells showed significantly decreased p-AMPK(Thr172) levels, consistent with inactivation of the Lkb1 kinase. Correspondingly, we also observed decreased phosphorylation of the AMPK target, acetylcoenzymeA carboxylase, indicating that Lkb1 deletion was associated with defective AMPK signaling in these tumors (Fig. 3A). Surprisingly, however, inactivation of Lkb1 in endometrial epithelium did not globally affect the phosphorylation status of ribosomal protein S6K (Fig. 3A), an important target of mTOR, and, indirectly, AMPK (2). Previous studies (using an antibody from the same source) have documented uniformly increased p-S6K levels in the epithelium of gastrointestinal polyps from Lkb1 mice (22). Although we did observe small foci of p-S6K–positive cells (Fig 3B, inset), this seems to be secondary to focal increases in mitotic rate because S6K is phosphorylated during mitosis (23) and p-S6K is also a well-documented marker of mitotic cells (24). We also did not observe significant alterations in the patterns or levels of phosphorylation of other putative mTOR substrates including p-4EBP1(Thr70) or p-Akt(Ser473) (data not shown). Next, Western blot analyses were done. Although the presence of stroma may have limited the sensitivity of this approach, significant decreases in p-AMPK were observed, whereas levels of total AMPK were unaffected. Furthermore, analysis of other mTOR pathway components was consistent with the in situ studies, including increased phosphorylation of S6K (Fig. 3A and B). Thus, although misregulated mTOR signaling has been reported in Lkb1+/−–associated gastrointestinal polyps (22), it seems that this is not a prime pathway by which Lkb1 loss promotes endometrial carcinogenesis.

Lkb1 tumors are extremely well differentiated without overt defects in cell polarity. At high magnification, Lkb1 tumors seemed to be extremely well differentiated, so much so that isolated tumor glands were virtually indistinguishable from normal endometrial glands (Fig. 4A). Although loss of polarity is characteristic of adenocarcinomas, Lkb1 tumor cells exhibited normal polarity in H&E-stained tissue sections and consisted of tall columnar cells with basally located nuclei and vacuolated secretory-type apical cytoplasm. Nuclear morphology was also not significantly altered, suggesting that these tumors are not highly aneuploid (Fig. 4A). These findings were surprising because tumors (Fig. 2C, peripancreatic tumor deposit). Mitotic and apoptotic counts were ascertained for uterine tumors relative to adjacent normal endometrial epithelium within the same mouse (n = 8). Data were plotted for individual mice because in randomly cycling females, endometrial cell growth/death vary through the estrus cycle, potentially obscuring aggregate analysis. In some tumors, mitotic activity and apoptotic index were significantly altered (Fig. 2D), consistent with the notion that Lkb1−/− endometrial tumor cells, despite their well-differentiated appearance, do exhibit abnormal responses to exogenous growth and survival stimuli.

Figure 4. Normal cell polarity in Lkb1 tumors from Ad-Cre–treated Lkb1−/− females. A, high magnification showing portion of a single gland, H&E-stained sections. Left, normal endometrium (em); right, tumor (tu). Tumor glands are indistinguishable from normal glands. Tall columnar tumor cells exhibit normal polarity with basal nuclei and apical secretory-type cytoplasm. Bar, 10 μm. B, lectin immunohistochemistry shows normal differentiation and expression on apical membrane in both normal endometrium and tumor (arrowheads; a single positive secretory vacuole is apparent in the tumor gland). Bar, 10 μm. C, ultrastructural analysis reveals normal apical differentiation including presence of abundant microvilli on apical cell surface. Morphology, distribution, and number of microvilli in tumor are similar to those of normal endometrium. Normal apical tight junctional complexes are also observed in tumor epithelial cells (white arrows). Images are representative of large areas scanned by electron microscope. Bar, 0.4 μm (both images at same magnification).
Lkb1 is required for the establishment of cell polarity in a variety of other cell types including gastrointestinal epithelium (2, 9). To further analyze potential defects in cell polarity, we used an antibody against a-lectin (Ulex Europaeus; ref. 25). Expression in tumors was confined to the apical surface, further arguing against gross polarity defects (Fig. 4B). Lastly, ultrastructural analysis also failed to reveal cell polarity defects. Tumor cells expressed numerous microvilli on apical, but not basolateral, surfaces, and

Figure 5. Low expression levels of Lkb1 in endometrial cancers correlate with invasiveness. A, validation of anti-Lkb1 antibodies in samples subjected to formalin fixation and paraffin embedding. 293T cells were transfected with a full-length human Lkb1 cDNA, trypsinized 24 h later, resuspended in a soft agar plug, and the solid plug was then fixed in 10% formalin overnight. Left, control untransfected cells; right, Lkb1-transfected cells. Antibody detects endogenous Lkb1 in untransfected control 293T cells by Western blot (not shown) and endogenous Lkb1 is also detectable as weak signal by immunohistochemistry (left). In transfected cells, a proportion of cells shows much higher signal (transfection is <100% efficient). Slides were counterstained with hematoxylin. Bar, 20 μm. B, representative Lkb1 expression levels categorized as high, intermediate, and low. Only staining in tumor cells (not stroma) was scored. A and B, bar, 20 μm; slides were counterstained with hematoxylin. C, Lkb1 expression in low-grade and high-grade endometrial cancers (n = 190). D, Lkb1 expression in stage 1A, 1B, and 1C endometrial cancers (n = 121). FIGO, International Federation of Gynecology and Obstetrics.
microvillous morphology and density were unaltered. Furthermore, no defects in other polarized structures such as tight junctions were observed in tumor cells (Fig. 4C).

Low LKB1 expression in human endometrial cancers correlates with invasiveness. Sequencing of LKB1 coding exons in 40 human endometrial tumors revealed a non–germ line mutation leading to a single conservative amino acid change (p.Arg331His) in only 1 of 40 human endometrial tumors. To determine if LKB1 down-regulation by other mechanisms might contribute to endometrial cancer progression, expression was assessed by immunohistochemistry of 190 endometrial cancers in a single tissue microarray. The antibody was first validated for LKB1 detection following formalin fixation/paraffin embedding of 293T cells with enforced LKB1 expression (Fig. 5A). Expression in primary tumor cells was classified as high, intermediate, or low (Fig. 5B). In high-grade versus low-grade tumors, a statistically significant increase in cases with low LKB1 expression was observed ($P = 0.035$, Fisher’s exact test; Fig. 5C). We then categorized expression by clinical stage. Stage 1A tumors are defined by lack of myometrial invasion, whereas 1B tumors invade through <50% of the myometrium and 1C tumors invade through >50% of the myometrium. LKB1 expression strongly correlated inversely with stage, with stage 1C tumors being much more likely to express low levels of Lkb1 than 1A tumors ($P = 0.0053$, Fisher’s exact test; Fig. 5D). These data suggest that LKB1 constrains invasion in vivo, consistent with our mouse studies. Furthermore, these findings suggest that down-regulation of LKB1, which may occur through a variety of mechanisms, is an important molecular event driving endometrial cancer progression. Our results also suggest that detection of LKB1 in primary tumors, as assessed by immunohistochemistry or other techniques, may aid in the identification of tumors more likely to be invasive and hence associated with a worse prognosis.

Discussion

It is striking that germ line or somatic inactivation of a single gene, Lkb1, provokes endometrial cancers in >50% of female mice. This high tumor incidence suggests that Lkb1 is, unexpectedly, a very potent endometrial tumor suppressor, and our finding that decreased expression correlates with invasiveness in human tumors is the first evidence that Lkb1 similarly drives human endometrial carcinogenesis. Another significant finding in our studies relates to the role of mTOR as a downstream target of Lkb1. The Lkb1-mediated repression of mTOR through the AMPK-Tsc pathway is thought to be important for tumor suppression, and the common regulation of mTOR by Tsc, Pten, and Lkb1 provides a unifying molecular explanation for hamartoma syndromes. The Lkb1-mutant endometrial tumors in our studies did not show overt mTOR dysregulation despite having defects in AMPK signaling. Hence, the Lkb1 tumor suppressor program in endometrium may not involve mTOR repression, or alternatively, there may be different mechanisms for malignant and benign tumor suppression. Among these lines, it is notable that Lkb1 deficiency promotes a highly invasive phenotype, whereas Pten inactivation promotes multifocal endometrial hyperplasias that become invasive only infrequently and with relatively long latency (26–31).

That only a subset of mice developed uterine tumors and the focal nature of these neoplasms suggest that cooperating genetic events are required for Lkb1-driven neoplasia. Loss of the second Lkb1 allele may not be requisite because some gastrointestinal polyps in Peutz-Jeghers syndrome patients or Lkb1−/− mice do not exhibit loss of heterozygosity (LOH) or loss of Lkb1 expression (2, 18). We similarly did not find consistent evidence of LOH in Lkb1−/− tumors, although the highly stromal makeup of these tumors may have obscured this analysis. We also note that many tumors invaded through the entire myometrium, yet remained confined to the uterus with no evidence of metastasis, suggesting that additional genetic events are required for extratubal spread. The nature of cooperating genetic events in Lkb1-driven neoplasia and their precise order in tumor initiation and progression remain important unresolved issues, but our mouse model should prove a useful tool to address these questions.

We show that Lkb1 functions as an endometrial cancer invasion gene, consistent with a recently described mouse model of lung cancer where Lkb1 deficiency conferred significantly increased mortality (32). Cells in most cancers exhibit multiple morphologic abnormalities (dedifferentiation, nuclear atypia, etc.) that are manifestations of growth signal self-sufficiency, invasion, and genomic instability (33). It is remarkable that Lkb1 endometrial cancers exhibit a relatively pure invasive phenotype with minimal dedifferentiation. The lack of gross polarity defects further suggests that Lkb1 inactivation can promote cancer through diverse, highly context-dependent mechanisms, and that the functions of Lkb1 in regulating invasion and cell polarity are separate and distinct. Along these lines, the absence of widespread S6K hyperphosphorylation in Lkb1 endometrial cancers (in contrast to gastrointestinal polyps in Lkb1 mice; ref. 22) lends further credence to the notion that Lkb1 promotes tumor growth via context-dependent mechanisms, with different downstream effectors varying in their significance in different tumor types.

We speculate that a variety of mechanisms may serve to down-regulate Lkb1 in human cancers; these may include promoter hypermethylation (5) or proteasomal degradation (34). Lastly, important parallels can be drawn between the endometrial tumors we describe in mice and the endocervical neoplasms observed in women with Peutz-Jeghers syndrome. The striking similarities of these tumors (extremely well-differentiated morphology, yet highly invasive) reveal fundamental similarities in Lkb1-driven carcinogenesis in the female reproductive tract in mice and women.

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