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INSIGHTS

Competence against insufficiency: Why are men mostly safe from a rare and deadly prostate cancer?

Grinu Mathew and Lloyd C. Trotman

Prostate cancer is a slow-growing disease, but not always. A highly rare and lethal form of the disease shows survival rates of less than a year. It is called squamous cell prostate carcinoma. In this issue of *JEM*, Hermanova et al. (https://doi.org/10.1084/jem.20191787) provide new findings in mouse demonstrating a strong genetic handle on both the reasons behind the rarity and the aggressiveness.

Defects in LKB1 kinase have been identified as the vulnerability behind Peutz–Jeghers syndrome, which is characterized by benign gastrointestinal hamartomas that predispose these patients to a higher risk of cancer incidence (Hemminki, 1999). This finding emphasizes the functional pathway relationship to the PTEN tumor suppressor as patients with PTEN hamartoma tumor syndrome suffer from related defects and cancer predispositions (Zbuk and Eng, 2007). In sporadic tumors, loss-of-function mutation of LKB1 kinase is most prevalent in non-small cell lung cancer at an ∼15% frequency (as curated at https://www.cbioportal.org). A recent report revealed the power of LKB1 mutation in causing an immune suppressive environment in non-small cell lung cancer patients that results in poor response to immune checkpoint inhibitor PD-1 (Skoulidis et al., 2018). Other cancer types with somatic mutations of LKB1 kinase are cervical carcinomas, pancreatic and biliary cancers, and melanomas.

The LKB1 kinase (encoded by the *STK11* gene) activates 14 known downstream targets that belong to the AMPK family of kinases (Shackelford and Shaw, 2009). AMPK itself is a major sensor for the low energy state of the cell. Upon sensing low nutrient status, the activated AMPK suppresses anabolic processes in favor of catabolic processes (Herzig and Shaw, 2018). This is the opposite behavior to that of mTORC1, which senses high nutrient status to promote anabolic processes. Thus the positive control of AMPK through LKB1 can be seen as a second tumor-suppressive restriction of mTORC1 because it acts in parallel to the PTEN-mediated restriction but under the control of nutrient status, not growth signaling as is the case for PTEN. Thus a major focus of studies on the tumor suppressor role of LKB1 pertains to this control over catabolism and mTORC1 via AMPK.

Given this prominent role in control of normal and tumor metabolism, there has been much interest in modeling the role of LKB1 status on the incidence or outcomes of cancer. Now, surprisingly, the report by Hermanova et al. in this issue of *JEM* suggests that LKB1 may play a major role in prostate cancer (PC)—not in the classic slow-growing epithelial adenocarcinoma, but instead in the highly lethal squamous cell PC (scPC). Equally intriguing, this may link scPC not to AMPK and metabolic control but instead to a set of lesser-known LKB1 targets: the SIK kinases, intriguing new players in cancer that strongly link LKB1 to transcriptional control (Wein et al., 2018).

The scPC was cataloged only a few decades ago due to its rarity. However, once diagnosed with this form of PC, there is a significant decrease in survival compared to epithelial PC, and only very few therapeutic options are available. scPC has a 32% chance of metastasis (Brunnhoelzl and Wang, 2018), which is five times that of adenocarcinoma. Furthermore, androgen deprivation therapy (ADT) shows little efficacy. Another important feature of scPC is that it can be treatment induced. Although it was originally identified as a rare entity, about half of the reported cases of scPC occur due to antihormone or radiation therapy of prostatic adenocarcinoma. With the advent of deep sequencing approaches, lineage switching and cellular plasticity have emerged as prominent features of treatment resistance in prostate adenocarcinomas. Treatment-induced emergence of squamous carcinoma can be caused by either de-differentiation of the cancer cell to a more stem-like progenitor state or by trans-differentiation to a squamous lineage that is now resistant to

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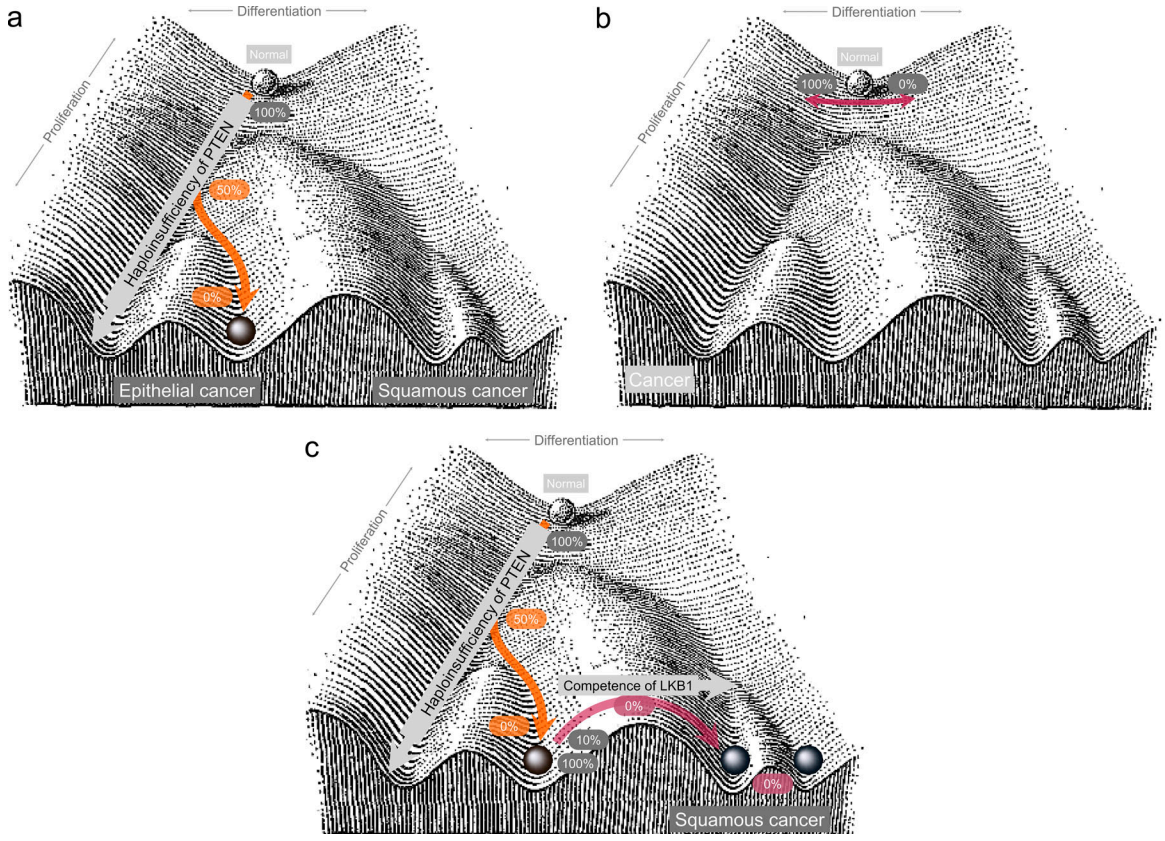
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Lloyd C. Trotman: trotman@cshl.edu.



Haploinsufficiency of PTEN in suppressing cell proliferation results in a spontaneous path on a downhill slope as PTEN levels decrease (a). Of note, this path toward PC is formed by a stable valley. Variable levels of LKB1 have little effect on normal prostate epithelial cells (b). After PTEN loss, there is a steep path for transdifferentiation from epithelial to squamous cancer (c). Partial loss of LKB1 will not suffice to escape from the epithelial valley. This gives rise to the concept of high LKB1 competence against transdifferentiation. Complete LKB1 deletion is required to push cells toward the squamous cancer lineage.

therapy (Labrecque et al., 2019). In treatment-naive cases displaying purely scPC, the disease is thought to originate from the urothelial lining or from the peri-urethral ducts. Another possible explanation for the de novo occurrence of this phenotype is the differentiation of pluripotent stem cells that can give rise to both adenocarcinoma and squamous forms of PC (Arva and Das, 2011).

In this issue of *JEM*, Hermanova et al. (2020) uncover an intriguing phenomenon. Adenocarcinoma of PC is successfully modeled in genetically engineered mouse (GEM) models by tissue-specific deletion of the *Pten* tumor suppressor in luminal epithelial cells (Arriaga and Abate-Shen, 2019). The result is a slow-growing tumor that typically remains indolent/prostate confined over the lifetime of the mouse. In contrast, they found that codeletion of *Pten* and *Stk11*, encoding Lkb1, resulted in aggressive tumors and lung metastasis, while prostatic deletion of Lkb1 on its own showed no phenotype. This kind of synergy between prostate tumor suppressor genes had previously been seen with *Pten*-loss in, for example, the *Pten*/*Trp53* or the *Pten*/Smad4 co-deletion mouse models. Loss of *Trp53* or loss of *Smad4* on its own shows no prostate phenotype, yet the codeletions with *Pten* display lethal PC. However, the Lkb1 (*Stk11*)/*Pten* double mutant prostate showed a highly unexpected result: instead of the widespread epithelial adenocarcinoma, the tumors that emerged were of the scPC type. Furthermore, these tumors showed a high frequency of lung metastasis, which is surprising for a germline-based GEM model both in terms of the frequency (>80%) and the apparent selective tropism of metastasis specifically to lung. Moreover, the lung metastatic lesions clearly retained the squamous cell phenotype as characterized by the positivity for cytokeratin 5 and the transcription factor p63, both markers that define the basal prostate cell compartment. This is in contrast to the epithelial *Pten*-deficient lung metastasis seen in two PC GEMs, which showed high concordance (Cho et al., 2014; Ku et al., 2017): lung metastatic nodules showed no p63 staining (nor was cytokeratin 5 seen in RapidCaP metastasis). Thus, the loss of LKB1 clearly generated a distinct entity of *Pten* mutant PC. The authors confirmed a 22% frequency in primary patient tumors with *LKB1* hemizygous loss. The analysis of metastatic disease and the frequency in scPC will require deeper analysis given the scarcity of samples.

A second surprising result was obtained. When testing the patient-derived kinase mutant of LKB1 (K78I), Hermanova et al. (2020) noted that it did not recreate the Lkb1 (*Stk11*)-null phenotype of proliferation and migration/metastasis in vitro and in transplantation models. This suggested that the 10% activity remaining in the LKB1 mutant is sufficient for normal LKB1 function in these cells. Since this stands in contrast to the known haploinsufficient and dose-to-function behavior when suppressing PTEN, the authors considered LKB1 to be highly "competent" for tumor suppression.

There are two plausible explanations for the switch from adenocarcinoma to scPC

upon dual loss of Pten and Lkb1: (1) A shift in cell-of-origin: from a luminal (*Pten*-KO only) to a basal cell (*Pten*/*Stk11* doubleKO); and (2) the trans-differentiation of luminal tumor cells into cells with basal/squamous character ($p63^+$, $Ck5^+$). While more work is needed to differentiate between these and other possible explanations, it is very tempting to speculate that LKB1 is involved in the prevention of a transdifferentiation step along the second hypothesis.

First, LKB1 loss has previously been shown to drive lineage switching in lung cancer. Using genetic experiments that are highly related to the ones presented in this issue of *JEM*, Wong and colleagues showed that Kras-driven lung adenocarcinoma switched to squamous cell carcinoma specifically after deletion of Lkb1 (Zhang et al., 2017). This was due to activation of a squamous cell program through suppression of PRC2-mediated chromatin regulation. A second line of evidence for a critical role of LKB1 chromatin regulation was recently published. Vakoc and colleagues found that LKB1 supports a lineage specific transcription factor that is essential for survival of acute myeloid leukemia cells. Intriguingly, they could demonstrate that the relevant LKB1 targets for this function are the SIK2 and SIK3 kinases (Tarumoto et al., 2018) which control HDACs and the cAMP-regulated transcriptional coactivators. Hermanova et al. (2020) indeed show SIK1/2/3 suppression after LKB1 loss. Together, these data could support the hypothesis that LKB1 is critical for blocking the transdifferentiation of epithelial derived PC into the squamous cell lineage.

In this light, we may be learning how PC prevents squamous transdifferentiation (see figure). Hermanova et al. (2020), found that only 10% LKB1 activity is still sufficient to retain LKB1 function. Thus, one can hypothesize that transdifferentiation requires complete loss of LKB1 and, as a consequence, that the bar for transdifferentiation is very high. This concept is summarized in the Waddington landscapes shown in the figure. PTEN is haploinsufficient for proliferation in prostate. This is represented by a downhill slope in a stable valley of epithelial cancer along the line of PTEN suppression (panel a). In contrast, *LKB1* perturbation on its own does not show a notable phenotype in prostate, depicted as swings along the horizontal (panel b). In the context of PTEN loss, there is also a very high bar for transdifferentiation of an epithelial to a squamous tumor giving rise to the notion of LKB1 competence in preventing squamous differentiation. Complete LKB1 deletion is required to push cells toward the squamous cancer lineage, as depicted in panel c.

Collectively, this would lead to the following postulate about PC suppression: the high frequency of epithelial tumors is due to PTEN haploinsufficiency for proliferation, and the low frequency of scPC is due to the LKB1 competence in blocking transdifferentiation.

The work raises important questions. What is the mechanistic link between LKB1 activity and p63 activation, and which p63 isoforms are activated? Definitive experiments on the transdifferentiation versus cell-of-origin hypothesis can be designed as has been done for lung epithelial to squamous cancer differentiation (Zhang et al., 2017). If SIK proteins are found to be critical in blocking transdifferentiation, it will be very important to understand how AR function relates to these transcription-regulating kinases. This could lead to a much better understanding of the ADT-induced scPC. Furthermore, it will be important to understand the extraordinary tropism of the LKB1 mutant metastatic cancer cells to lung. Finally, since ADT is generally not successful in squamous cell carcinoma, it will be important to define novel targets based on the results. Unlike the results from acute myeloid leukemia (Tarumoto et al., 2018), SIK or LKB1 inhibition would be expected to worsen the disease by promoting scPC. Therefore, it will be important to test if a prostate-specific vulnerability exists upon loss of LKB1 function. This could afford us with much-needed targets for patients suffering from this lethal variant of PC.

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