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Targeting the LKB1 Tumor Suppressor

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Abstract

LKB1 (also known as serine-threonine kinase 11, STK11) is a tumor suppressor, which is mutated or deleted in Peutz-Jeghers syndrome (PJS) and in a variety of cancers. Physiologically, LKB1 possesses multiple cellular functions in the regulation of cell bioenergetics metabolism, cell cycle arrest, embryo development, cell polarity, and apoptosis. New studies demonstrated that LKB1 may also play a role in the maintenance of function and dynamics of hematopoietic stem cells. Over the past years, personalized therapy targeting specific genetic aberrations has attracted intense interests. Within this review, several agents with potential activity against aberrant LKB1 signaling have been discussed. Potential strategies and challenges in targeting LKB1 inactivation are also considered.

Keywords

LKB1 (serine-threonine kinase 11, STK11); AMP-activated protein kinase (AMPK); tumor suppression; mutations; targeting therapeutics

INTRODUCTION

The *LKB1* gene, also known as serine-threonine kinase 11 (*STK11*), was first identified by Junichi Nezu of Chugai Pharmaceuticals in 1996 in a screen aimed at identifying new kinases [1]. The human *LKB1* gene has been mapped to chromosome 19p13.3. The gene spans 23 kb and is composed of nine coding exons and a noncoding exon [2]. *LKB1* encodes for an mRNA of 2.4 kb transcribed in the telomere-to-centromere direction [3]. LKB1 protein contains 433 amino acids (aa) in human and 436 aa in mouse. Its catalytic domain spans from aa49 to aa309 with a sequence not closely related to any known protein kinases [4]. LKB1 is broadly expressed in all fetal and adult tissues examined although at different levels [5]. LKB1 forms a heterotrimeric complex with two accessory subunits, Ste20-related adaptor protein (STRAD) and mouse protein-25 (MO25) [6–8], and acts as a constitutively active serine/threonine kinase, which phosphorylates 13 AMP-activated protein kinase (AMPK) family members [9–13]. *LKB1* is mutated in Peutz-Jeghers syndrome (PJS), a germline disease manifested by polyps in the gastrointestinal tract, mucocutaneous pigmentation, and a markedly increased risk of cancer [1–4]. Mutations of *LKB1* are also found in a variety of cancer patients without PJS, such as those with sporadic non-small cell lung cancer, ovarian and breast cancer, cervical cancer, and pancreatic cancer [14–24]. In addition to the critical role in cell bioenergetics regulation, LKB1 also bears multiple

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CONFLICT OF INTEREST

There is no conflict of interest to report.

cellular functions associated with embryo development, epithelial cell polarity, cell cycle arrest, DNA damage response, apoptosis, and the dynamics and maintenance of hematopoietic stem cells [19, 24–31].

THE BIOLOGICAL FUNCTIONS OF LKB1

Cell metabolism

About a decade ago, studies from three different groups established that LKB1 is the long-sought kinase that phosphorylates AMPK [9–11]. AMPK is a heterotrimeric enzyme complex consisting of a catalytic α subunit and regulatory β and γ subunits, and functions as a protein serine/threonine kinase [32]. The α subunit contains a typical serine/threonine kinase domain and a carboxy-terminal regulatory domain. The β subunit acts as a scaffold for binding the other two subunits and contains a glycogen-binding domain. The γ subunit contains four cystathionine- β -synthase (CBS) domains that play a role in binding to AMP, ADP, and ATP [24, 32, 33]. AMPK is activated under conditions of ATP depletion and elevation in AMP levels, e.g. glucose deprivation, hypoxia, ischaemia and heat shock [24, 32–34]. In addition, it is also activated by several hormones and cytokines such as adiponectin and leptin, and by the anti-diabetic drug metformin [33–38]. Phosphorylation of Thr 172 in the activation loop of AMPK is required for AMPK activation [33]. Among the kinases that can activate AMPK, LKB1 is the most important and well characterized upstream kinase [24, 32]. Once activated, AMPK phosphorylates and inactivates a number of metabolic enzymes involved in ATP-consuming cellular events including fatty acid, cholesterol and protein synthesis, and activates ATP-generating processes including the uptake and catabolism of glucose and fatty acids, thereby maintaining the cellular energy balance [39–44]. Via direct phosphorylation of substrates and indirect regulation of gene expression, activated AMPK may also regulate cell cycle, inhibit cell proliferation, maintain cell polarity, induce cell autophagy, and enhance cerebral amyloid- β clearance [25, 39, 44–47]. Thus, LKB1-AMPK signaling is a multi-tasking pathway that regulates cell metabolism and survival.

It has been proposed that LKB1 also regulates cellular growth by controlling another tumor suppressor, tuberous sclerosis complex (TSC) via the AMPK-dependent pathway [48, 49]. Under energy starvation conditions, LKB1 phosphorylates and activates AMPK, which directly phosphorylates TSC2, thereby enhancing its ability to switch off the mTOR signaling [50]. In addition, AMPK may also phosphorylate and inactivate one of mTORC1 complex components, Raptor, thereby suppressing synthesis metabolism [51]. By inhibiting mTORC1, AMPK not only down-regulates expression of ribosomal proteins, but also reduces expression of HIF-1 α and thus expression of the glycolytic enzymes and transporters required for the Warburg effect [52, 53]. Consistent with this, expression of HIF-1 α and many of its target genes is markedly up-regulated in mouse embryo fibroblasts (MEFs) deficient in either LKB1 or AMPK [52]. In LKB1 knockout cells, the mTOR-signaling pathway could not be suppressed under low cellular ATP conditions [52]. Furthermore, hamartomatous gastrointestinal polyps derived from LKB1 mutant mice displayed increased S6K activity, a major target of mTOR [52, 54]. These findings suggest that mTOR overactivation contributes to hamartomatous tumor growth upon LKB1 inactivation. Thus, the tumor suppressive activity of LKB1 involves the activation of the LKB1-AMPK pathway and its downstream targets. On the other hand, it is worth mentioning that under stress conditions, such as depletion of growth factors and nutrients, hypoxia, and de-adhesion, as well as oncogenic stress induced by deregulated Ras and Myc, AMPK can activate multiple pathways that maintain bioenergetics homeostasis to promote cell survival [36, 55, 56]. Thus, energy-sensing function of AMPK may play a conditional oncogenic role, which confers a survival advantage under selection pressure, contributing to cancer cell evolution and the rise of progressive cell populations [56, 57].

Apoptosis and cell cycle arrest

The role of LKB1 in apoptosis has been indicated in particular by studies showing an absence of apoptosis in polyps from patients with PJS [28]. In this role, LKB1 has been found to associate with p53 physically and to regulate specific p53-dependent apoptosis pathways [28]. In addition, LKB1 has been reported to interact with and phosphorylate PTEN (phosphatase and tensin homolog deleted on chromosome ten), another tumor suppressor that has lipid phosphatase activity and that inhibits cell proliferation and survival [58]. LKB1 has also been found to suppress the anti-apoptotic factors, such as STAT3, JNK, c-myc, k-ras, MAPK, and cyclooxygenase-2, and to inhibit cell survival [3, 59, 60]. This observation adds a new line of support to earlier findings showing that LKB1 inhibits cell cycle progression [25]. LKB1's putative downstream targets, such as Brg1, p21, and p27, have been suggested to mediate LKB1-dependent cell cycle arrest [25, 61, 62]. Recently, Scott et al. additionally showed that LKB1 down-regulates the expression of cyclin D1 [63]. They found that the protein levels of cyclin D, cyclin E, and cyclin A2 were increased in DLD1, a colorectal adenocarcinoma cell line expressing catalytically inactive LKB mutants [63]. These observations suggest that the tumor suppressive function of LKB1 may result from the inhibition of cell cycle progression.

Cell polarity

The role of LKB1 in epithelial polarity is associated with the phosphorylation of different members of the AMPK super family, including MARK/Par1 (MAP-microtubule affinity-regulating kinases/Par-1 in *Caenorhabditis elegans*), AMPK, and mammalian STE20-like protein kinase 4 (MST4). MARK/Par-1 kinases have been identified in diverse species, including yeast (KIN1, KIN2), fruit flies (Par-1), and mammals (MARK), and are essential for asymmetric cell division and the establishment of cell polarity [26, 27, 64–69]. It is believed that phosphorylation of MARK/Par1 kinases by LKB1 has been implicated in cell polarity regulation of LKB1 [26, 27, 67]. LKB1 induces apical brush border formation in intestinal cells by phosphorylating MST4, which then activates ezrin [26, 70, 71]. LKB1 was found to localize in the primary cilium and basal body, and result in increased AMPK phosphorylation at the basal body and inhibition of the mTOR pathway, which limits cell size [72]. In addition, E-cadherin regulates AMPK phosphorylation in polarized epithelial cells by controlling the localization of the LKB1 complex through binding to STRAD α [73]. AMPK is also required for tight junction (TJ) formation [27, 69, 74] although it is also possible that this activation of AMPK might be mediated by other upstream kinases, such as Ca²⁺-calmodulin-dependent protein kinase kinase- β (CAMKK β) [75–77]. AMPK regulates bile canalicular formation, TJ formation and polarity maintenance [78, 79]. Importantly, LKB1-deficient phenotypes in cell polarity regulation can be rescued by a phosphomimetic version of AMPK α . Thus, it seems that LKB1 signals through AMPK to coordinate epithelial polarity and proliferation with cellular energy status [26, 64, 80], and that AMPK and the MARK family members have overlapping substrates co-regulating epithelial polarity.

Mitosis

A genome-wide screen searching for mitotic regulators identified LKB1 as a protein kinase of interest and showed that down-regulation of LKB1 induces spindle aberrations [81]. The finding was recently confirmed by Wei et al. showing that loss of LKB1 causes changes in the angle of spindle orientation in an AMPK dependent manner, which may eventually lead to malfunction of mitosis [82]. Consistently, Banko *et al.* discovered that inactivation of AMPK induced pleiotropic defects in cell mitosis and induced S phase arrest [83]. AMPK regulates the protein phosphatase 1 regulatory subunit 12C (PPP1R12C), which binds to myosin regulatory light chain and 14-3-3 in order to dephosphorylate mitotic proteins for

mitotic exit, and is necessary for mitotic progression [83, 84]. Moreover, phosphorylation of AMPK α at Thr-172 was required for the association of AMPK with the centrosome, spindle poles, and mid-body during mitosis [82–84]. It was noted, however, that this process can be independent of LKB1 and likely promoted by Ca²⁺/calmodulin-dependent protein kinase kinase β (CaMKK β) [84]. Taken together, these findings suggest that LKB1 may be indeed involved in the regulation of mitosis in AMPK dependent or independent way.

Maintenance of genome stability

Genomic instability plays a critical role in tumorigenesis and correlates with the acquisition of malignant phenotypes [85, 86]. The most common reason for genomic instability is DNA damage. Endogenous sources of DNA damage can result from cellular metabolism or errors in DNA replication and recombination [85, 86]. Exogenous sources of DNA damage include ultraviolet (UV) light, X-rays, oxidative stress, and chemical mutagens [86]. Regardless of the source, the consequence is a variety of nucleotide modifications and DNA strand breaks. To combat the insults and maintain genomic stability, the cell has evolved a network of DNA repair processes referred to as the DNA damage response (DDR) [86]. The DDR is composed of sensors that continuously survey the genome for damaged DNA, transducers (mediators) that relay the signals, and effectors that receive these signals and orchestrate the repair process [86, 87].

Analysis of the LKB1 protein sequence and structure has shown that LKB1 Thr 363 (Thr 366 in mouse) lies in an optimal phosphorylation motif for the phosphoinositide 3-kinase-like kinases, such as DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated kinase (ATM), and ATM- and rad3-related kinase (ATR). These kinases act as DNA damage sensors, functioning upstream of DNA damage and mediating DNA repair [86, 87]. Moreover, Fernandes et al found from a GST pull-down assay *in vitro* that wild-type ATM displays a DNA damage-induced association with LKB1, BRCA1, and p53 [88]. Additionally, Alessi's group reported that the phosphorylation of LKB1 at Thr 363 (Thr 366) was triggered following the exposure of cells to IR and that DNA damage-activated ATM kinase mediated this phosphorylation [89]. Consistent with these findings, recent reports showed that AMPK is involved in IR- and ROS-induced DNA damage response [90, 91]. AMPK α 2 was recruited to DSBs in an LKB1-dependent manner. AMPK α 2 depletion impaired KU70 and BRM recruitment to DSB sites. LKB1 depletion induced the formation of chromosome breaks and radials [90]. These results suggest that LKB1-AMPK signaling may contribute to DNA damage repair and play a role in the maintenance of genome stability.

Anoikis and inhibition of tumor progression and metastasis

The importance of LKB1 in tumor suppression was recently further highlighted by its function in the repression of cancer invasion and metastasis [14, 17, 92–94]. In the *in vitro* studies, investigations demonstrated that LKB1 knockdown increases cell motility and invasiveness, and induces the expression of several mesenchymal marker proteins accompanied by the expression of ZEB1, a transcriptional repressor for E-cadherin and an inducer for epithelial-mesenchymal transition (EMT), which is a critical phenotypic alteration initiating the invasion and metastasis of cancer cells [14, 92–94]. Anoikis is a form of apoptosis that is triggered by poor contact between the cell and the extracellular matrix (ECM). Cancer cells may become resistant to anoikis and consequently display anchorage independent growth. It was found that LKB1 involves in p53-dependent anoikis by regulating salt inducible kinase (SIK1), an AMPK family member [95]. SIK1 was required for LKB1 to promote p53-dependent anoikis and suppress anchorage-independent growth and invasion. Intriguingly, Ng et al [96] recently analyzed gene expression profiling of *anoikis* resistant cells and showed that detachment results in the activation of AMPK.

Anoikis resistance strongly correlates with and is dependent on AMPK activation. AMPK-dependent mTOR complex-1 (mTORC1) blockade and inhibition of energy-demanding protein synthesis are critical for *anoikis* suppression, through mitigation of the metabolic defects induced by detachment [96]. The results implicate that AMPK-mediated mTORC1 inhibition and suppression of protein synthesis are a means for bioenergetic conservation during detachment, thereby promoting *anoikis* resistance. It is not clear whether the function of AMPK is regulated by LKB1. Since the investigations were performed in transformed AMPK deficient cells, it also remains unclear whether both AMPK α isoforms contribute to the phenotype. Considering AMPK acting as a “stress management” kinase and performing under a contextual condition, depending on the degree of AMPK activation, the particular AMPK isoforms present, and other processes activated in the cell, it is possible that modest activation of AMPK engages cell protective mechanisms resulting in oncogene-like activities, whereas increased magnitude or duration of stress could induce growth arrest or cell death exhibiting a tumor suppressor function.

In a range of mouse models and clinical analyses, *LKB1* inactivation is consistently found to be associated with aggressive invasive and metastatic growth. In the aforementioned LKB1-SIK1-p53 *anoikis* model [95], loss of *LKB1* promotes metastatic spread and survival of cells as micro-metastases in the lungs. In a K-ras driven mouse model of lung cancer, *LKB1* inactivation provided the strongest cooperation in terms of tumor latency and frequency of metastasis as compared with classic tumor suppressors such as *p53* and *p16* [14]. With integrative genomic and proteomic analyses, Wong group further identified that NEDD9, VEGF, SRC, and CD24 play a critical role in the promotion of LKB1 repression-induced metastasis [14]. In addition, loss of LKB1 activates lysyl oxidase expression via mTOR-HIF1 α axis and promotes lung cancer progress through extracellular matrix remodeling [97]. The molecular mechanism linking LKB1 depletion to metastasis was recently further generalized by Liu et al in melanoma model with LKB1 inactivation and K-Ras activation [92]. Loss of LKB1 in skin keratinocytes, gastrointestinal, and prostatic epithelium was also recently reported to promote the development of cancer, which was markedly accelerated by carcinogens DMBA (7,12-dimethylbenz(a)anthracene) and MNU (N-methylnitrosourea). The carcinogen-treated mice with LKB1 insufficient are prone to highly invasive squamous cell carcinomas of the skin that arose apparently *de novo* without progressing through an *in situ* (papilloma) stage [98]. Given the frequent mutation of *Hras* by DMBA, this further suggests that Ras-dependent signals and LKB1 loss might display a specific synergy that is selected for in tumor cells.

Consistently, Castrillon's group recently surveyed LKB1 expression in cervical and endometrial cancers and demonstrated that LKB1 mutations in primary cervical cancers are connected to accelerated disease progression and death [17], and decreased LKB1 protein expression in endometrial cancers correlates with a higher grade and stage [23]. Furthermore, the group identified a novel endometrial-specific gene, Sprr2f, and developed a Sprr2f-Cre transgene for conditional gene targeting within endometrial epithelium [22]. Thus, they generated an *Lkb1* conditional knock-out model in endometrial epithelium and produced a completely penetrant *Lkb1*-based mouse model of invasive endometrial cancer. Strikingly, female mice with homozygous endometrial *Lkb1* inactivation did not harbor discrete endometrial neoplasms, but instead underwent diffuse malignant transformation of their entire endometrium with rapid extrauterine spread and death, suggesting that *Lkb1* inactivation was sufficient to promote the development of invasive endometrial cancer [22]. In contrast, mice with heterozygous endometrial *Lkb1* inactivation only rarely developed tumors, which were focal and arose with much longer latency, arguing against the idea that *Lkb1* is a haploinsufficient tumor suppressor [22].

Hematopoietic stem cell (HSC) homeostasis

Three papers in the end of 2010 provided convincing evidence for the establishment that LKB1 is essential for the maintenance of hematopoietic stem cell (HSC) homeostasis [29–31]. Using mice in which LKB1 was conditionally deleted from hematopoietic tissues, researchers observed that loss of LKB1 leads to a decline in bone marrow cellularity, progressive pancytopenia and animal death due to the increased levels of apoptosis and autophagy in LKB1-deficient HSCs [29–31]. HSCs transiently increase in number, an effect associated with enhanced proliferation, and then markedly decrease. This suggests that LKB1 is necessary to maintain quiescence specifically in HSCs. In addition, it was shown that LKB1-deficient HSCs form fewer colonies than controls in culture; and that LKB1-deficient bone marrow shows a markedly decreased ability to repopulate the hematopoietic system of irradiated mice [29–31]. Many LKB1-deficient HSCs are aneuploidy and possess an enhanced expression of phosphorylated histone H2AX, a marker of DNA damage [31]. These findings establish an essential role for *Lkb1* in HSC maintenance, and show that, among blood cell lineages, the HSC population is particularly sensitive to depletion of LKB1. LKB1 deficiency may elevate the metabolic or genotoxic stress, thereby triggering hematopoietic stem cell death and exhausting.

The mechanism by which *Lkb1* regulates HSC homeostasis seems to be largely independent of its downstream effectors AMPK, mTORC1, and FoxO [29–31, 99]. Although LKB1 deficient HSCs display a loss of AMPK α phosphorylation and an increase in phospho-S6, demonstrating decreased AMPK activity and increased mTORC1 activity in the HSC compartment as expected, decreased AMPK activity does not account for the observed HSC defects, as administration of the AMPK activators metformin or A-769662 failed to rescue phenotypes exhibited by LKB1 mutant HSCs [29, 30]. In addition, AMPK-deficient HSCs failed to phenocopy LKB1-deficient HSCs [31]. Since enhanced mTORC1 activity results in HSC depletion [100], this pathway was also observed for potentially mediating the HSC phenotype in *Lkb1* mutants. However, administration of rapamycin, an mTORC1 inhibitor, failed to rescue HSC depletion or BM reconstitution. FoxO-deficient HSCs exhibit reduced survival and function due to impaired reactive oxygen species regulation; however, administration of the antioxidant N-acetyl-cysteine (NAC), also failed to rescue these phenotypes in *LKB1* mutants, demonstrating a FoxO-independent role for LKB1 [29–31]. Collectively, these data demonstrate that LKB1 regulates HSC quiescence through an AMPK-, mTORC1-, and FoxO-independent mechanism.

To pursue a mechanistic explanation for LKB1-regulated HSC quiescence and survival, Gan et al performed a transcriptome analysis of *LKB1* mutant HSCs [29]. It revealed enrichment for genes involved in the PPAR γ metabolic pathway, with down-regulation of PPAR γ coactivators *Pgc-1 α* and *Pgc-1 β* [29]. Consistent with PPAR γ 's role in mitochondrial biogenesis and function, defects in mitochondrial number and function were observed. LKB1 depletion also caused a reduction in mitochondrial membrane potential and reduced ATP levels in HSCs [29–31, 101] — a sign of decreased mitochondrial integrity. Thus, it seems that LKB1 balances proliferation and quiescence in HSCs by regulating mitochondrial function. However, the specific effectors of LKB1 in HSCs have yet to be defined. Whether LKB1 regulates HSC quiescence through the 12 other AMPK-related kinases or through another mechanism remains an important area of investigation. Indeed, in a recent report, Lai et al investigated LKB1 expression and functions in human embryonic stem cells maintained on human amniotic epithelial cells (hESCs(hAEC)) or on mitotically inactivated mouse embryonic fibroblasts (hESCs(MEF)) [102]. They found that knockdown of LKB1 in hESCs results in upregulation of pluripotency marker genes of Oct4 and Nanog, whereas downregulation of differentiation markers (Runx1, AFP, GATA, Brachyury, Sox17 and Nestin) [102]. LKB1 directly regulates the p21/WAF1 gene through promoter-binding

in hESCs. LKB1 expression may act as a negative regulator of a self-renewal in hESCs [102]. Taken together, current data suggest that LKB1 is an essential stem cell factor that promotes HSC (as well as hESCs) quiescence and maintenance, potentially through multiple mechanisms yet to be elucidated.

LKB1 MUTATIONS

To date, more than 250 different mutations in LKB1 have been identified in PJS patients and sporadic cancers according to the Sanger Institute Catalogue of Somatic mutations in Cancer website (<http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=STK11&start=1&end=434&coords=AA%3AAA>). Half of these are missense or nonsense mutations, which mostly lead to truncations of the catalytic domain and impair LKB1 catalytic activity. However, there are also a significant number of point mutations, which are located in the kinase domain and in the C-terminal noncatalytic region [3]. It was reported that germline mutations of *LKB1* occur in 80% PJS patients [103, 104]. In these patients, the most important associated health-related concern is the increased risk of cancer [105]. Gastrointestinal tumors are the most commonly diagnosed tumors in PJS patients, but the risk of developing cancer from other origins is also markedly higher, such as cancers from breast, pancreas, and gonad, etc. [105, 106]. Patients with sporadic cancers have also been screened for mutations in the LKB1 gene. Although tumor-specific LKB1 alterations have been identified in many tumor types, their frequency is relatively low with the exception of non-small cell lung cancer (NSCLC), gastrointestinal tract tumors, and cervical cancer. In NSCLC, 30% of the patients are reported to be LKB1 inactivated [4, 20, 106, 107]. Recent reports displayed that 20% primary cervical cancers possess somatically-acquired mutations of *LKB1* [17]. Deletion of *LKB1* and novel fusion transcripts resulting from the combination of truncated *LKB1* and its neighboring genes are also common in cervical cancer cells [16, 17]. These differences for the cancer distribution patterns between PJS and sporadic cancer in LKB1 mutation remain unclear. As cancer genome program becomes complete, the accurate LKB1 mutation pattern may be revealed. In the era of targeted therapies, it may be critical and desirable for precisely characterizing the aberrance of important genes, such as LKB1, for the selection of patients for future individualized treatments based on the presence of specific gene mutations [108].

TARGETING LOSS OF TUMOR SUPPRESSORS FOR CANCER THERAPEUTICS

Various hallmarks have been proposed to explain the complex nature of cancer at molecular, cellular, and pathological levels [109, 110]. Recent advances in omics are leading to a more complete picture for the alterations in the hallmarks, in particular at the molecular level. One vital advance is to apply genomics to identify mutations, both driver and passenger, present in human cancers. Using this information, targeted cancer therapeutic provides a conceptual framework and useful tool for arriving at drugs that will preferentially kill cancer cells relative to normal cells with more specificity. For example, application of drugs (antibodies) to selectively target the protein product of the BCR-ABL translocation in chronic myeloid leukemia (CML) has revolutionized the treatment of this disease, with five-year survival rates of 90% in treated patients [111]. For another example, p53 mutations have been found in most of the cancers. Mutated p53 may play a dominant negative role against wt-p53 partner and results in loss of functions of wt-p53, which increase tumor aggressiveness and metastatic potential [112, 113]. Strategies targeting mutant p53 have focused on destabilization or inactivation of mutant p53, or reactivation of wild-type function in the mutant p53 protein [114]. A newly characterized small molecular compound, PRIMA-1, can restore wild-type conformation of mutant p53 and specific DNA binding, consequently triggering apoptosis in tumor cells carrying mutant p53 [115–117]. Thus, PRIMA-1

possesses a preferential growth inhibitory activity on human cancer cells carrying mutant p53 relative to normal cells with wt-p53. This distinguishes PRIMA-1 from traditional anticancer drugs commonly used in treatment of malignant disease.

Mutations, such as p53, act as direct “druggable” objects, which can be targeted by various methods. There are another set of genetic mutations or depletions that although they are “drivers” for tumorigenesis, they may not be suitable as “druggable” targets themselves, or based on current knowledge or techniques, they cannot be targeted directly [118]. Thus, surveillance of the signaling pathways or interaction networks of the gene to explore the “synthetic lethality” may become a priority [118–122]. Synthetic lethality occurs when a single genetic inhibition does not harm a cell, but two genes simultaneously trigger death. In cancer, the oncogenic mutation disrupts the function of a single gene. If one can identify and disrupt the secondary pathway that is being up- or down-regulated compensating for the cancerous mutation, the cancer cells may die [123]. Since normal cells still have one functional pathway, they will remain unharmed. For example, oncogenic mutations and constitutive activation in the small GTPase Ras (K-Ras) are highly prevalent in cancer. However, K-Ras has not proven tractable as a drug target. Recently, two independent teams, led by Gilliland and Elledge respectively, identify two kinases - STK33 (serine/threonine kinase 33) and PLK1 (polo-like kinase 1) - in screens for synthetic lethality using short hairpin RNAs (shRNAs) in human cancer cells expressing mutant K-RAS [124, 125]. Luo et al [124] found that K-Ras mutant cells are hypersensitive to loss of the polo-like kinase PLK1, components of the anaphase-promoting complex/cyclosome, and the proteasome, whereas Scholl et al [125] demonstrated that inhibition of the protein kinases STK33 and TBK1 preferentially kills K-Ras mutant cells compared with K-Ras wild-type cells. In K-Ras mutant cells these kinases deliver critical pro-survival signals [121, 124, 125]. This work should spur interest in these kinases as potential therapeutic targets and also suggests a paradigm for synthetic lethal screening of human cancer cells in the future.

PARP family proteins (mainly PARP-1 and PARP-2) participate in the physiological response against DNA damage and repair of SSB-induced DNA damage [126, 127]. Lack of PARP activity with genetic modification or inhibitors increases SSB count. These unrepaired SSBs are converted into DSBs at fork replication. If cells are deficient in DSB repair, cells will be flooded with DSBs leading to cell death [128–130]. In most cell lines, treatment with PARP inhibitors at doses that successfully inhibit PARP activity does not cause cell death, in particular for cells with intact DSB DNA repair [127]. Until 2005, these agents were used in clinical trials as chemosensitizers independently of the DNA repair function. Therefore, there was great interest in the 2005 discovery that breast cancer cells bearing homozygous mutations in either the BRCA1 or BRCA2 cancer susceptibility genes were extremely sensitive to PARP inhibition [129, 130]. Investigation of the underlying mechanisms revealed that both BRCA1 and BRCA2 play important roles in the HR DNA repair pathway, and that continuous exposure of cycling cells to a PARP inhibitor resulted in the accumulation of DSB damage that could not be repaired [127–131]. The effect of PARP inhibitors was then extended to tumors with other genes implicated in similar DNA repair pathways to BRCA [132–135]. This abnormal function is called BRCAness and its clinical relevance was recently highlighted in triple-negative breast cancers [136–138].

Acknowledging that LKB1 bears multiple physiological functions and that LKB1 mutant cancers are biologically distinct from those with LKB1 intact [3], attentions turn to ways of targeting the mutations. Although progress has been made in the characterization of LKB1 mutations and its downstream signaling and identification of potential regulators controlling aberrant LKB1 downstream signaling, currently, there is no report for directly targeting LKB1 mutations. An alternative option may be through the targeting of downstream pathway components, such as inhibition of proliferation-associated proteins upregulated by

mutations of LKB1, such as mTORC1, or correction of disturbed cell metabolic signaling (e.g. glycolysis) observed in LKB1 and/or inactivation of AMPK [53]. These are attractive options, as some agents with activity against these targets are already in development (Table 1).

TARGETING LKB1 DEFICIENCY IN CANCERS

Agonists of AMPK

Given the multiple functions that LKB1-AMPK pathway bears in cell metabolism, AMPK has received a great deal of pharmaceutical interest as a target for type 2 diabetes and other aspects of the metabolic syndrome [38, 139–141]. For example, observations demonstrated that long-term treatment of genetically modified animal models of obesity or type 2 diabetes mellitus with the AMP analog 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), which activates AMPK, ameliorates these conditions by reversing hyperglycemia, hypertension, hypertriglyceridemia, and insulin resistance [11, 24]. AICAR also reduces hepatic glucose output, inhibits whole body lipolysis in diabetes patients, and stimulates glucose uptake in human skeletal muscle [33]. Thus, current evidence has suggested that AMPK may indeed be an ideal target for diabetes and metabolic syndrome and, thus, activation of AMPK may represent a significant focus for the development of next generation diabetes treatment.

On the other hand, activation of AMPK also imposes a metabolic checkpoint for the cell cycle through the phosphorylation of p53 and the inactivation of mTORC1 [142]. In mature tissues that do not require proliferation to maintain their functions, AMPK helps to keep the resting cell phenotype and protects cells from oncogene-induced transformation [143–145]. In proliferating cancer cells, AMPK activation switches off aerobic glycolysis, a process that cancer cells rely on for survival [53]. Thus, AMPK-activating drugs might be useful as cancer therapeutics. Indeed, for instance, many natural compounds previously found to suppress cancer growth with unknown mechanism could achieve their effects through activation of AMPK. Compounds, such as metformin and salicylate, are derived from composites found first in medicinal plants, and now they are shown to be AMPK activator [146–148]. More importantly, both compounds have been linked to lowered cancer risk. In a recent effort attempting to find specific AMPK direct activators, Abbott Laboratories developed a thienopyridone compound, A-769662, which was reported to allosterically activate AMPK independent of AMP binding [149]. Instead, binding of A-769662 depends in part on Serine 108 (S108) located in the carbohydrate binding domain of the β -subunit of AMPK [150]. Following A-769662 binding, S108 is autophosphorylated, resulting in the protection of the activating phosphorylation of T172 from upstream phosphatases, similar to the effect of AMP binding to the γ -subunit [151, 152]. It was reported that A769662 could effectively inhibit growth of multiple cancer cells both in vitro and in vivo [149–153]. The plausible interest for the exploration of A769662 is that it serves as a model for synthesizing more specific AMPK activator and that it provides a concept-proofing strategy demonstrating the feasibility for cancer therapeutic by specific activation of AMPK (Fig. 1).

Metformin is the most widely prescribed drug for type 2 diabetes and is thought to act by decreasing hepatic gluconeogenesis [37, 38]. Metformin and its more potent analogue, phenformin, inhibit complex I of the mitochondrial respiratory chain, resulting in reduced ATP production and LKB1-dependent activation of AMPK [140, 141]. Application of metformin leads to a dose-dependent reduction in breast cancer risk [154], which is believed to be mediated by the LKB1-AMPK-mTOR signaling [155, 156]. Consistently, inactivation of LKB1-AMPK pathway abrogates the inhibitory effects of metformin on cancer cells [157, 158]. In addition to the regulatory action of metformin on cell bioenergetics, metformin may also inhibit tumorigenesis via other mechanisms. For example, metformin induces cell cycle

arrest and promotes cell senescence by inhibiting cyclin D1 expression and pRb phosphorylation [159]. Metformin decreases the levels of epidermal growth factor receptor 2 (Her2) in breast and pancreatic cancer cells, thereby reducing the growth of the cancer cells [160–162]. Moreover, metformin has a systemic effect, improving insulin sensitivity and decreasing insulin levels, a beneficial effect that could also contribute to tumor suppression considering that insulin promotes cancer cell growth [158]. Hirsch et al recently reported that metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth by preferentially inhibiting nuclear translocation of NF- κ B and phosphorylation of STAT3 in cancer stem cells compared with non-stem cancer cells in the same population [163]. Thus, metformin acts to eliminate cancer-initiating stem cells to prevent the relapse of cancer [164].

To gain an insight into the possible role of metformin in cervical cancer, we recently investigated the sensitivity of cervical cancer cells with different LKB1 expression statuses to the treatment of metformin [165]. We found that metformin induces apoptotic and autophagic cell death in LKB1 intact cervical cancer cells by activation of AMPK and inhibition of mTOR. In contrast, cervical cancer cells with compromised LKB1 are relatively resistant to metformin. Overexpression of LKB1 reestablishes cellular sensitivity to metformin, suggesting that LKB1 is necessary for the response to metformin in cervical cancer cells [165]. LKB1 is a major kinase of AMPK. Activated AMPK mediates many functions of LKB1 [3, 24, 33, 38, 166]. Thus, it is reasonable to postulate that activation of AMPK in the context of LKB1 deficiency would be restrained under the treatment of metformin and other agonists [38, 166]. Although this is the case in most systems, several studies have found that AMPK is partially phosphorylated at Thr172 by CAMKK and TAK1 [75–77, 84], and thus, metformin may have an impact on LKB1-deficient cells. On the other hand, it is also possible that LKB1 may act through other targets independent of AMPK since LKB1 may phosphorylate additional 12 members of the AMPK kinase family [12, 96, 167]. Thus, more investigations are needed to determine the role of additional LKB1 substrates in mediating the cytotoxicity of metformin. In addition, Memmott et al recently applied metformin to tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced mouse lung tumorigenesis model and found that different tissues respond to metformin variously in terms of the activation of the molecular targets. In liver tissue, metformin activates AMPK and inhibits mTOR. In lung tissue, however, metformin does not activate AMPK but inhibits phosphorylation of insulin-like growth factor-I receptor/insulin receptor (IGF-1R/IR), Akt, extracellular signal-regulated kinase (ERK), and mTOR [147, 155]. It remains unknown whether LKB1 also plays a role in metformin-induced suppression of these kinases and whether metformin exerts a similar mechanism in other models.

Consistent with previous dispensable role of LKB1 mediating the effect of metformin on tumor suppression, Thomas's group recently demonstrated that biguanides inhibit mTORC1 signaling, not only in the absence of TSC1/2 but also in the absence of AMPK [168]. Along with these observations, in two distinct preclinical models of cancer and diabetes, metformin acts to suppress mTORC1 signaling in an AMPK-independent manner, indicating that at least partly, biguanides-induced anti-tumor effect is mediated by additional mechanisms [169, 170]. Consistent with this hypothesis, Thomas's group further characterized that the ability of biguanides to inhibit mTORC1 activation and signaling is dependent on the Rag GTPases [168]. Rag GTPases, Rags, are a novel family of GTPases activated by amino acids, stimulating mTORC1 signaling [171, 172]. The ability of Rag GTPases to mediate this response is based on their capacity to induce translocation of mTORC1 to a perinuclear intracellular compartment occupied by Rheb [172]. Similar to the effect of amino acid withdrawal, treatment of cells growing in complete media with phenformin caused mTOR to disperse throughout the cytoplasm. Although phenformin phenocopied amino acid

withdrawal, it had no effect on amino acid steady-state levels. Moreover, constitutively active, but not wild-type (WT), Rag GTPase protected mTORC1 signaling from inhibition by phenformin [168]. These data clearly demonstrated that Rag GTPases are targeted by biguanides although more detailed signaling pathway information triggered in the process and how many functions of biguanides are linked to the inhibition of Rag GTPases need further investigations.

Several groups added further evidence for the independence of LKB1 and AMPK in biguanides-mediated regulation of cell metabolism and cell growth. Foretz et al. reported that blood glucose levels in mice lacking AMPK in the liver are comparable to those in wild-type mice, and the hypoglycemic effect of metformin is maintained [170]. Metformin decreased expression of the gene encoding the catalytic subunit of glucose-6-phosphatase (G6Pase) in wild-type, AMPK-deficient, and LKB1-deficient hepatocytes. Metformin-induced inhibition of glucose production is amplified in both AMPK- and LKB1-deficient cells as compared with wild-type hepatocytes. This inhibition correlates in a dose-dependent manner with a reduction in intracellular ATP content, which is crucial for glucose production [170]. These results suggest that metformin-induced inhibition of hepatic glucose output is mediated by reducing cellular energy charge rather than direct inhibition of gluconeogenic gene expression. In another report, Miller et al [169] revealed that in mouse hepatocytes, metformin leads to the accumulation of AMP and related nucleotides, which inhibit adenylate cyclase, reduce levels of cyclic AMP and protein kinase A (PKA) activity, abrogate phosphorylation of critical protein targets of PKA, and block glucagon-dependent glucose output from hepatocytes. These data add another layer of evidence demonstrating the dispensability of AMPK and LKB1 in the sustenance of metformin as a cell metabolic regulator.

Consistent with the novel mechanisms of biguanides in cell metabolism regulation, their functions on tumor cell growth inhibition face alternative explanations as well. Applying LKB1 expression and host diet as variables, Algire et al [173] observed that metformin inhibits tumor growth and reduces insulin receptor activation in tumors of mice with diet-induced hyperinsulinemia, independent of tumor LKB1 expression. In the absence of hyperinsulinemia, metformin only inhibited the growth of LKB1 depletion tumors, a finding attributable neither to an effect on host insulin level nor to activation of AMPK within the tumor. Further investigation in vitro showed that cells with reduced LKB1 expression are more sensitive to metformin-induced adenosine triphosphate depletion owing to impaired ability to activate LKB1-AMPK-dependent energy-conservation mechanisms. Thus, loss of function of LKB1 can accelerate proliferation in contexts where it functions as a tumor suppressor, but can also sensitize cells to metformin due to a more vulnerable bioenergetic status in these cells [173]. This postulation was further proved recently by Shaw and colleagues using phenformin, a mitochondrial inhibitor and analog of metformin in NSCLC model [174]. They found that treatment with phenformin selectively triggered apoptosis in NSCLC cell lines lacking functional LKB1. In genetically engineered mouse models of NSCLC driven by oncogenic *Kras* and deficient in either *p53* or *LKB1*, ablation of *LKB1* blocked AMPK activation and enhanced apoptosis in lung tumors following phenformin treatment, leading to decreased tumor burden, delayed tumor progression, and prolonged survival in *Lkb1*-null mice compared with control and *p53*-deficient animals [174]. The preferential antitumor activity of phenformin in *Lkb1*-deleted tumors was associated with a greater decrease in intracellular ATP, decreased mitochondrial function, and increased mitochondrial reactive oxygen species levels, suggesting that mitochondrial defects in the absence of LKB1 enhance the sensitivity of NSCLC cells to phenformin [174]. Explanation for the preference is that *LKB1* mutation prevents activation of AMPK, which regulates cell growth and maintains energy homeostasis, leading to impaired cellular responses to metabolic stress. Phenformin inhibits mitochondrial complex I and continuously reduces

ATP levels, which could not be compensated in tumor cells with a nonfunctional LKB1-AMPK pathway due to their inability to respond to energy stress, thereby triggering cell death [174, 175]. LKB1 is mutationally inactivated in 20%~30% of NSCLC. Thus, this study suggests phenformin as a cancer metabolism-based therapeutic may selectively target LKB1-deficient tumors.

The conflicting data also warrant a need to re-validate or re-characterize the clinical application of biguanides in the clinic. It was noted that although population studies suggest that metformin exposure is associated with reduced cancer risk and/or improved prognosis, these data are mostly retrospective and nonrandomized [176]. Prospective and randomized trials should be emphasized. Statuses of signaling pathway molecules, such as LKB1, AMPK, mTOR, K-Ras, Rag GTPases, could be analyzed for better evaluating the therapeutic response to the biguanides. In addition, ongoing translational research should also be useful in guiding design of clinical trials, not only to evaluate metformin at conventional antidiabetic doses, where reduction of elevated insulin levels may contribute to antineoplastic activity for certain subsets of patients, but also to explore more aggressive dosing of biguanides, which may lead to reprogramming of energy metabolism in a manner that could provide important opportunities for synthetic lethality through rationalized drug combinations or in the context of genetic lesions associated with hypersensitivity to energetic stress.

mTOR inhibitors

mTOR is the catalytic subunit of two distinct complexes named mTOR complex 1 (mTORC1) and mTORC2. Components of mTORC1 include regulatory-associated protein of mTOR (RAPTOR), a negative regulator, 40 kDa Pro-rich Akt substrate (PRAS40; also known as AKT1S1) [177], whereas mTORC2 contains rapamycin-insensitive companion of mTOR (RICTOR), protein observed with RICTOR 1 (PROTOR1) and PROTOR2, which are likely to help complex assembly, and mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1), which may target mTORC2 to membranes [39, 177, 178]. mTORC1 and mTORC2 share mammalian lethal with SEC13 protein 8 (mLST8; also known as GβL) and the recently identified DEP domain-containing mTOR-interacting protein (DEPTOR), which function as positive and negative regulators, respectively [177–179]. Biochemical and structural evidence suggests that both mTORC1 and mTORC2 may exist as dimers [177]. When active, mTORC1 phosphorylates the translational regulator eukaryotic translation initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1), which, in turn, promote protein synthesis [180, 181]. Through the phosphorylation of several other effectors, mTORC1 promotes biogenesis and metabolism and suppresses autophagy by integrating nutrient signals that are generated by amino acids, growth factors such as insulin and insulin-like growth factors (IGFs), energy signals that act through AMPK and various stressors including hypoxia and DNA damage [39, 182–184]. Several groups recently reported that mTORC1 affects gene transcription and regulates the activation of transcription factors [39, 184]. The activity of mTORC1 towards certain substrates is very sensitive to the macrolide rapamycin. When bound to the 12 kDa FK506-binding protein (FKBP12), rapamycin physically interacts with and suppresses mTORC1 kinase activity [182]. Compared with mTORC1, less is known about mTORC2 [39, 108, 183, 184]. Because of its role in phosphorylating and activating Akt, mTORC2 forms a core component of the phosphoinositide 3-kinase (PI3K) pathway. When active, mTORC2 regulates cell survival, metabolism and cytoskeletal organization through the phosphorylation of several members of the AGC kinase subfamily [39, 177, 184]. Because the activity of mTORC2 is not blocked by acute treatment with rapamycin, this complex was originally described as the rapamycin-insensitive mTOR complex [185, 186]. However, a

recent report showed that chronic treatment with rapamycin may disrupt mTORC2 integrity and confer insulin resistance in experimental animals [187].

mTOR is a central node intergrading different cell signals to regulate cell growth. LKB1 phosphorylates and activates AMPK, which switches off mTOR through phosphorylation of TSC2 (tuberin) or through direct action on mTORC1 component, raptor [51]. PTEN, another tumor suppressor, inhibits mTOR through inactivation of AKT [188]. Thus, inactivating mutations in genes that negatively regulate the mTORC1 pathway, such as LKB1, PTEN, TSC1, TSC2, increase mTOR activation, which further phosphorylates a number of downstream substrates including proteins involved in the regulation of protein translation such as S6K1 and 4E-BP1 [39, 184, 188]. Among the mRNAs known to be translationally up-regulated by mTORC1 are a number of key pro-growth proteins including cyclin D1, cyclin D3, Mcl-1, c-myc, and the hypoxia inducible factor 1 alpha (HIF-1 α) [52]. Therefore, activation of mTORC1 de-represses protein synthesis, and promotes cell growth, proliferation and tumorigenesis. On the other side of the story, dysregulation of mTOR in tumors with these genes deficiency provides an opportunity for the development of individualized targeted therapies.

mTOR inhibitors have been used as a targeted agent in preclinical studies and clinical trials. Either alone or in combination with other conventional anticancer agents, mTOR inhibitors have the potential to provide anticancer activity in numerous tumor types, including renal cell carcinoma (RCC), neuroendocrine tumors, leukemia, lymphoma, hepatocellular carcinoma, gastric cancer, pancreatic cancer, sarcoma, endometrial cancer, breast cancer, and non-small-cell lung cancer, etc. [108, 189, 190]. Rapalogues including temsirolimus, everolimus, and ridaforolimus (formerly deforolimus) have been assessed for their safety and efficacy in cancer patients. Temsirolimus has been approved by FDA for intravenous application in the treatment of advanced renal cell carcinoma. Everolimus (rapamycin) is an oral agent that has recently obtained FDA approval for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib and also for breast cancer [189–192]. Ridaforolimus remains an investigational targeted agent in clinical development and not yet approved for any indication. Several other mTOR inhibitors are under development.

Given that inactivating mutations in the *PTEN*, *NF1*, *TSC2* or *LKB1* tumor suppressor genes lead to cell-autonomous hyperactivation of mTORC1, ultimately resulting in tumors that are reliant on mTORC1 signaling, special interests are focused on administration of mTORC1 inhibitors in inherited cancer syndromes or hamartomas with these aberrations [24, 182, 189]. In recent clinical trials, rapamycin and its analog temsirolimus were shown to have palliative success in clinical trials on patients with PTEN-deficient glioblastomas and metastatic renal cell carcinoma [24, 52]. Furthermore, in a pair of phase II clinical trials involving tuberous sclerosis (TSC) and lymphangiomyomatosis (LAM) patients, partial responses to the rapamycin analog Sirolimus were observed, including regression of angiomyolipomas with continuous therapy [193, 52], consistent with previous clinical observations in TSC patients given rapamycin [52]. Combined with data from mouse models, these clinical data suggest that hamartoma syndromes with hyperactivation of mTORC1 may be particularly responsive to rapamycin analogs as a single agent.

Consistent with this concept, rapamycin was administrated in genetically engineered mice with LKB1 conditional knock-out. Rapamycin monotherapy in LKB $^{+/-}$ mice with established polyposis led to decreased polyp burden and size [52, 54, 194–196]. Rapamycin treatment initiated prior to the onset of polyposis also led to a dramatic reduction of polyp size and overall burden [196], suggesting that rapamycin is effective in both treatment and prophylaxis in a faithful animal model of PJS polyposis. Two groups recently evaluate the impact of LKB1 in gynecology tumors. Contreras et al found *LKB1* inactivation is sufficient

to drive endometrial cancers. Rapamycin monotherapy not only greatly slowed disease progression, but also led to striking regression of pre-existing tumors [22]. These studies not only demonstrate that *LKB1* is a uniquely potent endometrial tumor suppressor, but also suggest that the clinical responses of some types of invasive cancers to mTOR inhibitors may be linked to *LKB1* status. Tanwar et al. conditionally deleted *Lkb1* in mouse Müllerian duct mesenchyme-derived cells of the female reproductive tract and observed expansion of the stromal compartment and hyperplasia and/or neoplasia of adjacent epithelial cells throughout the reproductive tract with paratubal cysts and adenomyomas in oviducts and, eventually, endometrial cancer [197]. mTORC1 activation was found in stromal cells of both the oviduct and uterus. Loss of *PTEN* along with *Lkb1* deletion significantly increased tumor burden in uteri and induced tumorigenesis in the cervix and vagina. Treatment with rapamycin decreased tumor burden in adult *Lkb1* mutant mice [197]. These studies show that *LKB1/TSC1/TSC2/mTORC1* signaling in mesenchymal cells is important for the maintenance of epithelial integrity and suppression of carcinogenesis in adjacent epithelial cells. Because similar changes in the stromal population are also observed in human oviductal/ovarian adenoma and endometrial adenocarcinoma patients, the authors speculated that dysregulated mTORC1 activity by upstream mechanisms similar to the described model systems may also contribute to the pathogenesis of these human diseases and indicate a beneficial effect for rapamycin in this kind of patients [194, 197].

It is worth mentioning that although much effort has been devoted to the study of mTOR inhibitors, including rapamycin and its analogs, in the treatment of cancer, particularly as the side effects associated with these agents have been relatively mild, the clinical results have been different with cancers from various tissues [177, 182]. Rapalogs appear to be cytostatic and improve survival primarily by stabilizing disease in some patients, and to be marginal response to others [182]. This may be due to *S6K1*-mediated feedback loop. Rapamycin only partially suppresses mTORC1 function, efficiently inhibiting *S6K1* but not *eIF4E*; thus, it only partially blocks translation [177]. Moreover, owing to the inhibition of the *S6K1*-dependent feedback loops, rapamycin indirectly upregulates *PI3K* activity to promote cell survival [177, 182]. In addition, *S6K1* inhibition activates the *MEK-ERK* signaling cascade, as well as transcription of platelet-derived growth factor receptor (*PDGFR*) [178, 179, 184, 195]. These trigger feedback loops to counteract the action of rapamycin, dampening its effectiveness in cancer models and in patients [179]. Taking advantage of this compensatory pathway (activation pathway), dual *PI3K-mTOR* inhibitors designed to block both mTOR and *AKT* pathways have the potential to achieve synergistic effects if the toxicity of the combination therapies is manageable [24, 39, 184]. In addition, recently developed ATP-competition based mTOR catalytic inhibitors target all known functions of mTORC1 as well as mTORC2; thus, they inhibit translation more potently. Although *PI3K* overactivation still occurs, Akt phosphorylation by mTORC2 (feedback effort under rapamycin treatment) is impaired [182].

Heat shock protein 90 (Hsp90) inhibitors

HSP90 is a molecular chaperone, which is upregulated in response to stresses. It regulates and stabilizes a number of key proteins, including *PI3K*, *AKT*, *EGFR*, and wild-type and mutant *p53*. Hsp90 requires the presence of co-chaperone proteins to enable it to interact specifically with its client proteins [198]. One of these co-chaperone proteins is *Cdc37*, which specifically targets Hsp90 to a variety of protein kinases. Hsp90 stabilizes its target proteins and prevent their degradation by the proteasome complex [199, 200]. A significant number of oncogenes, such as the *ErbB* receptor [201], *p210^{bcr-abl}* and *v-Src* [202], as well as the mitogen-regulated *MOK* kinase [203], are rapidly degraded following treatment of cells with Hsp90 inhibitors such as geldanamycin.

To date, there are 17 distinct Hsp90 inhibitors in clinical trials for multiple indications in cancer [198, 204–206]. These inhibitors have shown limited single-agent activity, but more promising clinical efficacy has been seen when combined with other drugs. One fundamental principle for the combination administration is that Hsp90 inhibitors play a unique role in preventing drug resistance in tumors because oncogenes rely heavily on Hsp90 to chaperone their otherwise unstable conformation due to their mutations [198]. This dependence has been termed oncogene addiction [207]. Because numerous mutant oncoproteins are ‘addicted’ to Hsp90 activity, an inhibitor to Hsp90 has the ability to affect multiple targets and pathways, which can prevent oncogene switching, a major mechanism for developing resistance [208, 209]. For example, Paraiso et al. recently investigated the potential use of the HSP90 inhibitor (XL888) in different cell models of vemurafenib resistance. XL888 inhibited tumor growth and induced apoptosis in vemurafenib-resistant melanoma cell lines [210]. HSP90 inhibition was shown to be more effective in restoring BIM (apoptosis inducer) and down regulating Mcl-1 (prosurvival protein) than combined MEK/PI3K inhibitor therapy. HSP90 inhibition may be a highly effective strategy at managing the diverse array of resistance mechanisms being reported to BRAF inhibitors [210].

LKB1 was originally found to be associated with Hsp90 chaperone and the co-chaperone Cdc37 by two groups [211, 212]. It was demonstrated that Cdc37 and Hsp90 bind specifically to the kinase domain of LKB1. However, Hsp90 and Cdc37 interact with both wild-type and kinase-dead LKB1, indicating that the catalytic activity of LKB1 is not required for its association with these proteins [211]. Consistent with the key role of Hsp90 in regulating the stability of LKB1, treatment of cells with Hsp90 inhibitors markedly lowered LKB1 protein levels. Treatment of cells with either geldanamycin or novobiocin, two pharmacological inhibitors of Hsp90, causes LKB1 destabilization with geldanamycin treatment leading to ubiquitination and rapid degradation of LKB1 by the proteasome-dependent pathway [212]. In the early reports, it was revealed that Hsp90/Cdc37 does not directly influence the intrinsic LKB1 catalytic activity [211]. In a recent following-up study, Gaude et al. further characterized the interaction between HSP90-CDC37 and LKB1 and described the dual activities of the HSP90–CDC37 chaperone machinery in maintaining the stability while inhibiting the activity of LKB1 kinase [213]. Disruption of the LKB1-Hsp90 complex favors the recruitment of both Hsp/Hsc70 and the U-box dependent E3 ubiquitin ligase CHIP (carboxyl terminus of Hsc70-interacting protein) that triggers LKB1 degradation [213]. LKB1 in complex with HSP90–CDC37 has a longer half-life but is incapable of autophosphorylation, and its kinase activity is increased upon HSP90 inhibition [213]. These results establish that the Hsp90-Cdc37 complex controls both the stability and activity of the LKB1 kinase.

Interestingly, an LKB1 point mutation (G163D) identified in a sporadic testicular cancer weakens the interaction of LKB1 with both Hsp90 and Cdc37 and enhances its sensitivity to the destabilizing effect of geldanamycin [212, 213]. Considering mounting evidence for LKB1 mutations in tumorigenesis and progression, enhancing the degradation of inactive LKB1 may warrant a selective therapeutic choice for LKB1 mutation cancers. It remains unknown whether other LKB1 mutations are also more sensitive to HSP90 inhibitors than the wild-type LKB1 and the significance of the destabilization of the mutant LKB1 also needs to be further validated since whether these LKB1 mutants play a dominant negative role in vivo remains unclear. Consistently, a recent cancer genetic-based drug screening profiled the sensitivity of cancer cell lines with various genetic abnormalities to 130 different anticancer agents [214]. In the analysis, inactivation of LKB1 was statistically associated with sensitivity to the HSP90 inhibitor 17-allylaminogeldamycin (17-AAG) although the gene–drug association was not expanded and the nature of LKB1 aberrations was not defined [214]. However, the association does indicate the potential of HSP90

inhibitors in the application of LKB1 deficient individuals and merits further investigation. On the other hand, due to the stabilization of LKB1 by the HSP90-CDC37 complex [211–213], Hsp90 inhibitors may induce degradation of wild-type LKB1, a point deserving careful attention. In line with this consideration, determination of LKB1 status may be needed prior to HSP90 targeting therapy.

Cyclooxygenase-2 (COX-2) inhibitors

Cyclooxygenases catalyze the synthesis of prostaglandins and thromboxane from arachidonic acid. Two COX isozymes, COX-1 and COX-2 with 60% homology in humans, have been identified. COX-1, constitutively expressed in most tissues, mediates physiological responses and regulates renal and vascular homeostasis. COX-2, is considered to be an inducible immediate-early gene product whose synthesis in cells can be up-regulated by mitogenic or inflammatory stimuli including TNF- α , IL-1 β and lipoteichoic acid [215]. COX-2 is thought to be responsible for the production of pro-inflammatory prostaglandins (PGs) in various models of inflammation [216]. The COX-2 pathway has been shown to be involved in many processes leading to tumor progression such as angiogenesis, survival, proliferation, invasion, and immunosuppression [217]. COX-2 has been implicated in multiple malignancies, such as colorectal, esophageal, lung, breast, pancreas, and prostate cancers [218, 219]. In *Apc*^{Δ716} mouse, a model for familial adenomatous polyposis (FAP), researchers demonstrated that disruption of the gene encoding COX-2 or prostaglandin E₂ (PGE₂) receptor EP2 suppresses intestinal polyposis [220–222]. These results indicate that PGE₂ produced through the COX-2 pathway plays an important role in intestinal tumorigenesis. A similar observation was found in mutations of genes, whose aberrations are associated with gastrointestinal tumorigenesis [223].

Peutz—Jeghers syndrome (PJS) with LKB1 mutations is typically manifested as severe gastrointestinal polyposis and higher risk of developing gastrointestinal cancers [1–3, 224]. Interestingly, increased COX-2 expression was found in 60–80% of the polyps and all carcinomas from PJS patients [225, 226]. COX-2 expression was noted in the epithelial cells of hamartomatous polyps, and distributed throughout the stromal tissue of the lamina propria, including muscle cells [227]. Over-expression of COX-2 in PJS hamartoma and carcinoma samples is believed to be associated with resistance to apoptosis, thus increasing the tumorigenic potential [28]. Consistently, COX-2 was also significantly up-regulated in the polyps developed in LKB1 knockout mice [59]. COX-2 expression is often an early change and could thus have a significant impact on the further development of these tumors [28, 59, 224–228]. A dramatic reduction in large polyps and total tumor burden in a *Cox-2*-deficient background as well as following celecoxib treatment demonstrates a tumor promotion role for COX-2 in *Lkb1*^{+/-} mice [225, 229]. A similar role for COX-2 has been demonstrated in the *Apc*^{Δ716} mouse model of familial adenomatous polyposis [230]. The precise signaling mechanism mediated the elevation of COX2 in LKB1 insufficient individuals is largely unknown [3, 231, 232]. The complexity of COX-2 regulation has been underscored in studies of familial adenomatous polyposis coli patients and corresponding mouse models where the mechanisms by which adenomatous polyposis coli mutations elicit COX-2 induction remain elusive [233, 234]. COX-2 is induced by a wide spectrum of growth factors and pro-inflammatory cytokines through several signal transduction pathways including Rac1/cdc42/MKK/p38MAPK, Ras/MEKK/SEK/JNK, PI-3K/Akt, and Ras/Raf-1/MEK/Erk [235, 236]. Analysis of the mediators of these pathways in murine *Lkb1*^{+/-} polyposis revealed that only Erk1/2 was activated; Rossi et al found that the Ras/Raf-1/MEK/ERK signal transduction pathway is likely to mediate COX-2 induction in murine *Lkb1*^{+/-} polyposis [59]. Nevertheless, these findings indeed identify COX-2 as a potential target for chemoprevention and treatment of PJS patients and LKB1 deficiency-associated gastrointestinal tumors.

Inhibition of cyclooxygenase enzymes with nonsteroidal anti-inflammatory drugs has proven effective in repressing gastrointestinal tumorigenesis both in the general population and in patients with familial adenomatous polyposis coli [215, 216]. In particular, COX-2 has emerged as the central target of these therapies as shown by clinical trials using selective COX-2 inhibitors [216]. The findings that COX-2 expression is up-regulated in a significant percentage of PJS polyps have prompted the investigators to test COX-2 inhibitors as chemopreventive therapy in PJS patients and LKB1 conditional knock-out mice. Makela and colleagues have reported that treatment of *LKB1*^{+/-} mice with celecoxib, a COX-2 inhibitor, reduced polyp burden by over 50% [229]. Celecoxib treatment initiating before polyposis (3.5–10 months) led to a dramatic reduction in tumor burden (86%). Late treatment (6.5–10 months) also led to a significant reduction in large polyps [229]. This is encouraging in view of the clinical situation in which the disease is typically noted through large obstructing polyps. COX-2 inhibition decreased mean vessel density (MVD) in the polyps, which is similar to the FAP mouse model following COX-2 suppression [229, 237]. This correlation could be either because of a decreased need for vessels when polyps are of smaller size or because of a primary decrease in vascularity limiting the further growth of the polyps. The latter model would suggest that COX-2 may promote angiogenesis in polyps of *Lkb1*^{+/-} mice through up-regulation of VEGF and FGF as noted in FAP [237, 238]. In a pilot clinical study performed by the same group, a subset of PJS patients responded favorably to celecoxib with reduced gastric polyposis [229]. These data not only establish a role for COX-2 in promoting Peutz–Jeghers polyposis, but also suggest that COX-2 chemoprevention may prove beneficial for PJS patients.

In a most recent investigation, Makela and colleagues evaluated the protective effect of celecoxib on gastrointestinal polyposis in *Lkb1*^{+/-} mice aggravated by N-methylnitrosourea (MNU) [239]. Again, treatment with celecoxib is sufficient to improve the disease outcome in the *LKB1*^{+/-} mice even at a low dosage. However, they found that celecoxib did not suppress the polyposis in MNU-treated *LKB1*^{+/-} mice to the normal levels of *Lkb1*^{+/-} polyposis, suggesting that celecoxib therapy may not by itself be efficient in suppressing Peutz–Jeghers polyposis in settings where the polyposis is accelerated by additional mutations. Intriguingly, inhibition of COX-2 by inhibitors suppresses intestinal polyposis in several other tumor models or patients, such as trefoil factor 1 (TFF1)-deficient mice, *Apc*^{Δ716} mice, and FAP patients [220, 240, 241]. Thus, these results suggest that COX-2 induction in the tumor stroma is independent of the molecular mechanism that initiates tumorigenesis in the epithelial cells. In the future, it is important to further characterize which genetic settings influence cellular sensitivity to COX-2 inhibitors and which signaling pathways dominate the sensitivity to COX-2 inhibitors.

Inhibitors of Src, MEK, and PI3K

The proto-oncogene c-Src (Src) encodes a nonreceptor tyrosine kinase, the expression and activity of which are correlated with cancer progression, advanced malignancy, and poor prognosis in a variety of human cancers [242]. Nine members have been identified in the Src family kinases (SFKs). SFKs interact directly with receptor tyrosine kinases, G-protein-coupled receptors, steroid receptors, signal transducers and activators of transcription, and molecules involved in cell adhesion and migration, and facilitate the phosphorylation signals through *Ras*/*Raf*/*Erk1/2*, *PI3K*/*AKT*, focal adhesion kinase (FAK)/*p130CAS*/*paxillin*, and *c-myc*/*cyclin D1* [243, 244]. SFKs regulate a variety of biological functions including proliferation, cell growth, differentiation, cell shape, motility, migration, angiogenesis, and survival [242]. Recent data show that SFKs also regulate cancer progression and metastasis by action on divergent signals [242, 243]. Due to the role of Src in the integrity of various signaling pathways mediating different functions in cancer initiation and progression, Src is becoming a compelling therapeutic target for cancer treatment [242, 245]. Src inhibition has

been shown to decrease proliferation, microvessel density, and deduce metastasis in mouse models [242, 243, 246]. SFK-directed tyrosine kinase inhibitors (TKIs) possess the potential to apply as single-agent therapy in some malignancies [242, 246]. However, most Src family kinases have been shown to have limited single-agent activity in the clinical setting, therefore, combination of Src inhibitors with other chemotherapeutics is intensively investigated [242, 243, 244]. In addition, it is also important to further explore the acting molecular mechanisms of Src and to define the critical factors contributing to the successful clinical implementation of these inhibitors.

In a recent interaction target screening, Carretero et al found that loss of LKB1 works synthetically with SRC and FAK to enhance NSCLC progression and metastasis [247]. Cancer genomic studies have previously established a number of oncogene and tumor suppressor pathways as important for the initiation and maintenance of neoplastic lesions in NSCLC [248, 249]. However, the molecular alterations necessary for invasion and metastasis of NSCLC are less defined. The authors' group previously reported that deletion of *LKB1* in the context of *Kras*-driven murine lung tumors promotes invasion and metastasis [14]. To identify altered signal transduction pathways involved in the progression and metastases of LKB1-deficient lung tumors, Carretero et al compared gene expression and phosphoproteome profiles between primary *Kras*G12V tumors and primary *Kras*G12V/*Lkb1*^{-/-} tumors as well as metastatic *Kras*G12V/*Lkb1*^{-/-} tumors [247]. Loss of LKB1 in the primary tumor resulted in increased expression of genes associated with the FAK/SRC and PI3K/AKT pathways. In addition to these pathways, metastatic tumors showed increases in epithelial-mesenchymal transition (EMT), stem cell, and growth factor pathways [247]. Similarly, LKB1 loss in vitro also resulted in SRC activation, increased motility, and SRC-dependent adhesion [247]. More importantly, migration was selectively abrogated by SRC-family kinase inhibitor Dasatinib or the FAK inhibitor PF573228 in LKB1-deficient cells. Furthermore, whereas *Kras* mutant lung tumors are sensitive to the combined inhibition of the PI3K and MEK pathways, *Kras/Lkb1* tumors are resistant to these inhibitors, and that sensitivity can be restored by additional targeting of SRC [247, 249, 250], demonstrating the importance of SFKs in tumor growth and promoting resistance to combined PI3K/MEK inhibition.

Consistent with the role of LKB1 loss in tumorigenesis and progression that is promoted by Src, Liu et al recently showed that *Lkb1*-deficient melanoma cells increased invasive behavior in vitro compared to isogenic *Lkb1*-competent melanoma cells [92]. *Lkb1* loss and *K-Ras* activation develop highly penetrant melanomas that are extraordinarily metastatic [92]. Further investigation revealed that LKB1 deficiency resulted in activation of SFKs, particularly the Yamaguchi sarcoma viral oncogene homolog 1 (YES) in the SFK family, and expansion of a highly invasive and tumor-clonogenic subpopulation of cells expressing high levels of CD24, a modulator of metastasis and a marker of stem-progenitor cells *in vitro* and *in vivo* [92]. Importantly, inhibition of YES activity with shRNA or SRC-family kinase inhibitor Dasatinib suppressed CD24 expression and markedly decreased metastatic behavior, demonstrating that *LKB1* functions as a strong suppressor of melanoma metastasis by regulating YES activity. These findings provide further evidence for the therapeutic targeting of Src in LKB1 deficient cancers.

Consistently, before the Src-promoted LKB1 deficiency-induced phenotype was revealed, two reports had already showed that *LKB1* is functionally inactivated by activating mutations of *B-RAF V600E*, which are found in approximately 50% of human melanoma [251, 252]. Zheng et al. noticed that AICAR could not activate AMPK in B-RAF V600E melanoma cells but does activate AMPK in wild-type B-RAF cells. ERK and RSK, two kinases constitutively activated downstream of B-RAF V600E, phosphorylate LKB1 on S325 and S428, respectively, thereby compromising the ability of LKB1 to bind and activate

AMPK [251]. Furthermore, expression of LKB1 mutated at these phosphorylation sites allows activation of AMPK and inhibits melanoma cell proliferation and anchorage-independent cell growth, suggesting that suppression of LKB1 function by B-RAF V600E plays an important role in B-RAF V600E-driven tumorigenesis [251]. In another report, Esteve-Puig et al found that RAS pathway activation including B-RAF V600E mutation promotes the uncoupling of AMPK from LKB1 and protects cells from metabolic stress-induced apoptosis [252]. Notably, In both studies, inhibition of the RAF-MEK-ERK signaling with MEK inhibitors (U0126, PD98059, or CI-1040) in B-RAF V600E mutant melanoma cells recovered the LKB1/AMPK complex formation and rescued the LKB1-AMPK α metabolic stress-induced response, increasing apoptosis in cooperation with the pro-apoptotic proteins Bad and Bim, and the down-regulation of Mcl-1 [251, 252]. Considering the contextual role of Src (YES) in RAF-MEK-ERK signaling [92, 251–253], these reports show a reciprocal verification of each other, assure the importance of the Src-RAF-MEK-ERK signaling pathway in LKB1 loss-induced tumor progression, and provide a therapeutic target for LKB1 deficiency cancers.

Chk1 inhibitors and DTYMK suppression

LKB1 has been shown to play an important role in the maintenance of hematopoietic stem cell quiescence [29–31]. Deletion of *Lkb1* resulted in an initial expansion of HSCs and multipotent progenitor cells. With time, however, a depletion of these cell populations and eventually a depletion of all blood cell types (pancytopenia) occurred [29–31]. One of the explanations for the depletion of hematopoietic stem cell is that *Lkb1* deficiency leads to increased DNA damage in response to metabolic and genotoxic stresses. Gurumurthy et al. observed enhanced expression of phosphorylated histone H2AX, a marker of DNA damage, in hematopoietic tissues of LKB1-deficient mice, indicating that DNA damage may exist in LKB1 deficient cells [30]. Consistently, a recent study showed that LKB1-AMPK signaling regulates non-homologous end joining (NHEJ)-associated DNA repair and contributes to genome stability [90]. Thus, it seems true that LKB1 plays a role in DNA damage response. As a corollary, LKB1 deficient cells may be more sensitive to agents that promote DNA damage as compared with wild-type counterparts.

Consistent with this ideal, in a recent study, Wong and colleagues employed an integrative approach to define novel therapeutic targets in *Lkb1* mutant lung cancers and established that checkpoint kinase 1 (CHK1) inhibitor may act with LKB1 deficiency leading to synthetic lethality [254]. CHK1 is a DNA damage check point protein, which coordinates DNA repair signals to the cell cycle machinery to prevent progression or induces apoptosis [255]. ATR phosphorylates CHK1 on serines 317 and 345 resulting in CHK1 autophosphorylation on serine 296. Activated CHK1, in turn, phosphorylates the Cdc25A protein phosphatase to promote its ubiquitin-mediated proteolysis, which results in cell cycle arrest in the S- and G2-phases of the cell division cycle [256]. CHK1 also phosphorylates RAD51, FAND2 and FANCE to activate DNA repair pathways [257]. Thus, Chk1 is essential for DNA repair. Inhibition of CHK1 lessens cell cycle arrest under DNA damage and enhances damaged DNA in cycling cells. In addition, CHK1 inhibition also causes an increased initiation of DNA replication, which is accompanied by increased amounts of nonextractable replication protein A (RPA), formation of single-stranded DNA, and induction of DNA strand breaks [255]. Making use of the characteristic of CHK1 inhibition, several CHK1 inhibitors are currently undergoing clinical trials as anti-neoplastic agents [257–259]. These inhibitors are used largely in combination with other DNA damaging agents including cisplatin, fluorouracil, topotecan, and cytarabine [257–259]. In the profiling exploration performed by Wong and colleagues, matched cell lines from genetically engineered mouse models of cancer driven by activated K-Ras alone or in combination with LKB1 deletion, were employed in high-throughput RNAi, kinase inhibitor, and metabolite

screens [254]. These screens identified knockdown of CHK1 as synthetically lethal with LKB1 deficiency in both mouse and human lung cancer cell lines [254]. They further validated the observation by showing that LKB1-deficient H2122 and A549 were more sensitive than LKB1-wt H358 and Calu-1 cell lines to the treatment with the selected CHK1 inhibitors, AZD7762 and CHIR124. Moreover, this pathway appears relevant *in vivo* since LKB1 loss was associated with elevated CHK1 expression in K-Ras-mutant NSCLCs [254]. Thus, the results convincingly reveal that LKB1 inactivation confers a marked sensitivity to treatment with CHK1 inhibitors.

In the same report, Liu et al. identified another even more interesting target associated with LKB1 deficiency, DTYMK (deoxythymidylate kinase) [254]. With the similar screen strategy, they found that knockdown of DTYMK is synthetically lethal with LKB1 deficiency in both mouse and human lung cancer cell lines. DTYMK is the first enzymatic step following the convergence of the *de novo* and salvage pathways in dTTP biosynthesis. DTYMK catalyzes the phosphorylation of dTMP to form dTDP [260]. The production of dTDP is different from that of the other deoxyribonucleotides used in DNA synthesis—dADP, dGDP, dCDP, and dUDP, which are synthesized from ADP, GDP, CDP, and UDP by ribonucleotide reductase [260]. Thus, the unique dTTP biosynthesis pathway makes itself a distinctive target for drug development. Previous inhibitors for key enzymes in the *de novo* dTTP synthesis pathway, such as 5-fluorouracil, pemetrexed, and hydroxyurea, have verified the feasibility of the concept [261]. The global metabolite profiling in the report demonstrated that LKB1-null cells had striking decreases in multiple nucleotide metabolites as compared to the LKB1-wt cells [254]. Depletion of DTYMK in mouse and human NSCLC cells diminished the dTDP pool and led to greater growth inhibition in *LKB1*-deficient cells; and that LKB1 loss in mouse and human linked to more DNA damage [254]. One possible explanation for the synthetic lethality of *LKB1* loss with *Dtymk* knockdown is in part because of the lower expression of DTYMK in *LKB1*-null cells, leading them to be more dependent on the dTTP synthesis pathway. Knockdown of *Dtymk* depletes the absolute amount of DTYMK protein below a critical threshold, resulting in thymine-less death in *LKB1*-null cells but not in *LKB1*-wt cells. Taken together, although a complete mechanism by which DTYMK suppression triggers LKB1-compromised cell death remains elusive, these studies clearly suggest that DTYMK is a potential therapeutic target in *LKB1*-mutant human cancer. In the future, more efforts will be needed to decode the role of LKB1 in the regulation of DTYMK and to develop specific inhibitors of DTYMK.

ISSUES REMAINED TO BE SOLVED

AMPK is a well-characterized target of LKB1. While many functions of LKB1 are ascribed to the activation of AMPK [56], the roles of other 12 substrates in the mediation of LKB1 functions remain largely unknown. In the future, more efforts are needed in this regard in order to gain more information relevant to the general understanding of cancer and to provide more specific targets for LKB1-directed therapeutics as many of aforementioned strategies are indirect.

LKB1 inactivation has been broadly found in NSCLCs and cervical cancers. However, in terms of the biological functions in patients, whether there are any differences between inactive mutations and deletions of LKB1 are unknown. It is important to characterize the difference in the future for developing selective drugs structurally targeting the mutants if a dominant negative role indeed exists for some LKB1 mutants. In the latter case, the targeting drugs should also be evaluated for not affecting the endogenous wild-type LKB1 cells. For LKB1 deleted individuals, on the other hand, specifically targeting deregulated LKB1 downstream signals will be a research alternative.

As already applied by Wong group and others [92, 247, 254, 262], administration of comprehensive approaches integrated genomics, proteomics, metabolomics, and bioinformatics, may help identify genes or agents synergizing with Lkb1 loss and leading to synthetic lethality. A similar strategy could be used for other types of tumors with LKB1 deficiency.

Loss of LKB1 may affect multiple signals to induce tumorigenesis, disease progression, and metastasis. Thus, combination of agents targeting different pathways may generate maximum beneficial effect. Again, this is dependent on a thorough understanding of the biological functions of LKB1.

Heterogeneity of human cancer may become a hurdle for the selection of patients and obscure synthetic lethal associations for individualized treatments based on the presence of specific gene mutations, such as LKB1 aberration. Thus, in the coming years, the development of novel technologies that allow accurate molecular diagnosis of heterogeneous cancer specimens will be a major challenge. This point may also apply to the identification of other gene mutations and enable effective discovery of genotype-driven sensitivities.

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LIST OF ABBREVIATIONS

4E-BP1	Translation initiation factor 4E (eIF4E) binding protein 1
ACC	Acetyl-CoA carboxylase
AICAR	5-aminoimidazole-4-carboxamide-1- β -d-ribofuranoside
AMPK	AMP-activated protein kinase
ATM	Ataxia telangiectasia mutated kinase
ATR	ATM- and rad3-related kinase
BRCA1/2	Breast cancer susceptibility gene-1/2
CaMKKβ	Ca ²⁺ /calmodulin-dependent protein kinase kinase β
CBS	Cystathionine- β -synthase
CDC25	Cell division cycle 25
CHK1	Cell cycle checkpoint kinase 1
CML	Chronic myeloid leukaemia
COX-2	cyclooxygenase-2
DDR	DNA damage response
DMBA	7,12-dimethylbenz(a)anthracene
DNA-PK	DNA-dependent protein kinase
DTYMK	Deoxythymidylate kinase
ECM	Extracellular matrix

eIF4E	Translation initiation factor 4E
EMT	Epithelial-mesenchymal transition
Erk	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
FAP	Familial adenomatous polyposis
FKBP12	12 kD FK506-binding protein
FoxO	Forkhead-box protein O
Her2	Epidermal growth factor receptor 2
HIF-1α	Hypoxia inducible factor 1 α
HSC	Hematopoietic stem cell
HSP90	Heat shock protein 90
IGF-1R/IR	Insulin-like growth factor-I receptor/insulin receptor
JNKs	c-Jun N-terminal kinases
LKB1	Liver kinase B1
MARK	MAP-microtubule affinity-regulating kinase
MAPK	Mitogen-activated protein kinase
MEF	Mouse embryonic fibroblast
MNU	N-methylnitrosourea
MO25	Mouse protein 25
MVD	Mean vessel density
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex-1
mTORC2	mTOR complex-2
NAC	N-acetyl-cysteine
NF1	Neurofibromatosis Type 1
NHEJ	Non-homologous end joining
NSCLC	Non-small cell lung cancer
PAK1	p21-activated kinase 1
PGE2	Prostaglandin E2
PARP-1	Poly (ADP-ribose) polymerase 1
PDGFR	Platelet-derived growth factor receptor
PI3K	Phosphoinositide 3-kinase
PJS	Peutz-Jegher syndrome
PKA	Protein kinase A
PLK1	Polo-like kinase 1
PPAR-γ	Peroxisome proliferator-activated receptor gamma

PGC-1	Peroxisome proliferator-activated receptor-gamma coactivator 1
PPP1R12C	Protein phosphatase 1 regulatory subunit 12C
PRAS40	40 kDa Pro-rich Akt substrate
PROTOR1/2	Protein observed with RICTOR 1/2
PTEN	Phosphatase and tensin homolog deleted on chromosome ten
RAPTOR	Regulatory-associated protein of mTOR
RICTOR	Rapamycin-insensitive companion of mTOR
RCC	Renal cell carcinoma
RPA	Replication protein A
S6K1	S6 kinase 1
SFKs	Src family kinases
shRNA	Short hairpin RNA
SIK1	Salt inducible kinase
siRNA	Small interfering RNA
STAT3	Signal transducer and activator of transcription 3
STK11	Serine/threonine kinase 11 (also known as LKB1)
STK33	Serine/threonine kinase 33
STRAD	Ste20-related adaptor protein
TBK1	TANK-binding kinase 1
TKIs	Tyrosine kinase inhibitors
TSC	Tuberous sclerosis complex
TJ	Tight junction
UV	ultraviolet
WT (wt)	Wild type
YES	Yamaguchi sarcoma viral oncogene homolog 1
ZEB1	Zinc finger E-box binding homeobox 1

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Fig. 1.
Agonists regulate LKB1-AMPK signaling and its functions.

Table 1

Summary of experimental agents targeting signals resulting from LKB1 mutations

Class of agent	Specific agents tested	Action mechanisms	Properties of action
Agonists of AMPK	AICAR	<ul style="list-style-type: none"> AMP analog 	<ul style="list-style-type: none"> activate AMPK with LKB1 ameliorate metabolic syndrome induce apoptosis in LKB1^{-/-} cells
	2-DG metformin phenformin	<ul style="list-style-type: none"> inhibit glycolysis inhibit mito. respiratory chain low insulin decrease Her2 eliminate cancer stem cells inhibit Rag GTPases low cAMP 	<ul style="list-style-type: none"> inhibit tumor cell growth and metabolism inhibit growth of tumor cells with LKB1 from multiple tissues in vitro and in vivo preferentially kill LKB1 deficient cells by deteriorating bioenergetics synergy with additional therapy drugs
	A769662 Salicylate	<ul style="list-style-type: none"> allosterically bind and activate AMPK 	<ul style="list-style-type: none"> activate AMPK-mediated cell metabolism and delay tumor onset
mTOR inhibitors	temsirolimus, everolimus (rapamycin), ridaforolimus	<ul style="list-style-type: none"> interact with and suppress mTORC1 kinase activity inhibit mTORC1-mediated biogenesis and metabolism induce autophagy and apoptosis; regulate transcription factors and transcription 	<ul style="list-style-type: none"> LKB^{+/+} mice with rapamycin monotherapy treatment and prophylaxis in a animal model of PJS polyposis slow and regress <i>LKB1</i> inactivation driven endometrial, cervical, and vaginal cancers dual PI3K–mTOR inhibitors achieve synergistic effects
Hsp90 inhibitors	17-AAG geldanamycin novobiocin XL888	<ul style="list-style-type: none"> interfere with the ATP-binding domain of Hsp90 restore apoptosis and suppress prosurvival proteins potentiate the actions of anti-cancer drugs that target Hsp90 client proteins, prevent cancer drug resistance 	<ul style="list-style-type: none"> lead to the ubiquitination and the rapid degradation of both wt- and point mutation (G163D) LKB1 increase LKB1 kinase activity upon HSP90 inhibition inactivation of LKB1 sensitizes cells to 17-AAG
COX-2 inhibitors	Celecoxib Other non-steroidal anti-inflammatory drugs	<ul style="list-style-type: none"> inhibit COX-2-mediated tumor progression decrease mean vessel density in the polyps inhibit activated Ras/Raf-1/MEK/ERK signal in murine <i>Lkb1</i>^{+/-} polyposis 	<ul style="list-style-type: none"> reduced polyp burden (size and number) in <i>LKB1</i>^{+/-} mice prevent polyposis in <i>LKB1</i>^{+/-} mice a subset of PJS patients responded favorably to celecoxib with reduced gastric polyposis partially suppress the polyposis in MNU-treated LKB1^{+/+} mice

Class of agent	Specific agents tested	Action mechanisms	Properties of action
Src inhibitors	Dasatinib	<ul style="list-style-type: none"> inhibit Src-mediated tumor progression and metastasis sensitize other chemotherapeutics 	<ul style="list-style-type: none"> selectively abrogate tumor cell migration in LKB1-deficient cells restore the sensitivity of <i>K-Ras</i>-mutant/LKB1 loss tumors to PI3K and MEK inhibitors suppress CD24 expression and decrease metastasis of LKB1 loss melanoma
Chk1 inhibitors	AZD7762 CHIR124	<ul style="list-style-type: none"> Enhance DNA damage Sensitize cells to traditional DNA damaging agents synthetic lethality with LKB1 deficiency 	<ul style="list-style-type: none"> Chk1 is elevated in <i>K-Ras</i>-mutant/LKB1 loss NSCLC mice knockdown of Chk1 is synthetically lethal with Lkb1 deficiency in NSCLC LKB1-deficient H2122 and A549 are more sensitive than LKB1-wt H358 and Calu-1 cells to Chk1 inhibitors