

## Review

# The molecular mechanisms that underlie the tumor suppressor function of LKB1

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**Germline mutations of the *LKB1* tumor suppressor gene result in Peutz–Jeghers syndrome (PJS) characterized by intestinal hamartomas and increased incidence of epithelial cancers. Inactivating mutations in *LKB1* have also been found in certain sporadic human cancers and with particularly high frequency in lung cancer. *LKB1* has now been demonstrated to play a crucial role in pulmonary tumorigenesis, controlling initiation, differentiation, and metastasis. Recent evidences showed that *LKB1* is a multitasking kinase, with great potential in orchestrating cell activity. Thus far, *LKB1* has been found to play a role in cell polarity, energy metabolism, apoptosis, cell cycle arrest, and cell proliferation, all of which may require the tumor suppressor function of this kinase and/or its catalytic activity. This review focuses on remarkable recent findings concerning the molecular mechanism by which the *LKB1* protein kinase operates as a tumor suppressor and discusses the rational treatment strategies to individuals suffering from PJS and other common disorders related to *LKB1* signaling.**

**Keywords** *LKB1*; tumor suppressor; Peutz–Jeghers syndrome; lung adenocarcinoma

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## Introduction

The *LKB1* gene was discovered only in 1998 as a new tumor suppressor gene by linkage analysis of Peutz–Jeghers syndrome (PJS) [1,2]. PJS is a rare autosomal-dominant polyposis disorder that was first described in a Dutch family by Peutz in 1921 [3] and later by Jeghers *et al.* in 1949 [4]. The disease is characterized by the presence of hamartomatous polyps in the gastrointestinal tract and by melanin spots on the lips, buccal mucosa,

and digits [3,4]. Gastrointestinal tumors are the most commonly diagnosed malignancies in PJS patients, but the risk of developing cancer from other origins, including breast, intestine, testis, cervix, and pancreas, is also significantly high [5–10]. In 1998, groundbreaking genetic linkage analysis undertaken by two independent groups revealed that germline mutations of the *LKB1* gene, which is located on chromosome 19p13.3, are the cause of the majority of PJS cases [1,2]. In most previous studies, *LKB1* mutations proved to be detectable in 35–70% of PJS patients [11–13]. However, in these studies, screening methodologies for identifying *LKB1* mutations in PJS were mostly limited to detect point mutations and small-scale intra-exonic deletions/insertions. It was supposed that the prevalence of *LKB1* mutations in PJS was underestimated in these reports. The recently developed multiplex ligation-dependent probe amplification assay for PJS allows a systematic search for large deletions in the *LKB1* gene. Using this technique, three research groups found whole gene or exon deletions of *LKB1* account for 39% [14], 32% [15], and 50% [16] of the patients in whom no point mutation had been detected or 14%, 16%, and 24% of all patients examined, respectively. According to these studies, the total percentage of *LKB1* inactivation in PJS patients ranged from 66% to 94% [14–16]. Consistent with this finding, de Leng and colleagues [17] have recently found that 91% of the studied families showed *LKB1* inactivation, which included 78% point mutations and 13% exon deletions or even whole gene deletions.

Besides germline mutations of the *LKB1* gene are responsible for PJS, genetic alterations of *LKB1* have also been found to play a causal role in certain sporadic human cancers [18], especially in lung cancer [19–21]. Remarkably, most of the observed cancers are associated with somatic sequence mutations accompanied by loss of heterozygosity of the *LKB1* gene, which lead to the

generation of truncated and, therefore, inactive LKB1 proteins [22,23]. What is more, the truncated transcript of *LKB1* is significantly down-regulated and the truncated LKB1 protein is virtually undetectable in certain of sporadic cancer cell lines [23–25]. Promoter methylation accounts for a small fraction of repressed *LKB1* expression cases [24,25], but this is unlikely a major contributing mechanism in non-small cell lung carcinoma cell lines [23]. Additionally, homozygous deletions of *LKB1* have been observed in sporadic pancreatic and biliary adenocarcinomas [26]. It is suggested that biallelic inactivation of *LKB1* could favor progression to malignancy [22]. Although somatic *LKB1* alterations are rare in sporadic cancers, there are at least one-third sporadic lung adenocarcinoma harbor somatic mutations at the *LKB1* gene [19–21]. More recently, Ji *et al.* [6] have demonstrated that LKB1 plays a crucial role in pulmonary tumorigenesis, controlling initiation, differentiation, and metastasis. It is evident from the recent reports that LKB1 is a master kinase that can potentially activate several downstream kinases by phosphorylating a conserved threonine in their activation loops [27], among which partitioning defective gene 1 (Par-1)/microtubule affinity-regulating kinases (MARKs) and AMP-activated protein kinase (AMPK) are two kinases that have been extensively characterized [28–33]. LKB1 can also phosphorylate and activate 13 protein kinases from the AMPK family [27]. Therefore, LKB1 is a multitasking kinase, with great potential in orchestrating cell activity. The pathogenic mechanism of LKB1 in PJS polyps and cancers has been discovered and studied for 10 years; however, it has not been fully understood. Thus far, LKB1 has been found to play a role in cell polarity, energy metabolism, apoptosis, cell cycle arrest, and cell proliferation [34–38]. Therefore, characterization of the diverse signaling networks modulated by LKB1 can provide intriguing new insight into the molecular mechanism by which the LKB1 protein kinase operates as a tumor suppressor.

## Characteristics of the *LKB1* Gene and Encoded Protein

The *LKB1* gene (also known as serine/threonine kinase 11, *STK11*), mapping to the chromosomal region 19p13.3, is the first serine/threonine kinase gene whose inactivating germline mutations cause a cancer susceptibility syndrome [33]. It spans 23 kb and is made up of nine coding exons and a final non-coding exon [1]. *LKB1*

encodes for an mRNA of ~2.4 kb transcribed in telomere-to-centromere direction and for a protein of 433 amino acids and ~48 kDa [1]. The protein, which has serine–threonine kinase activity, possesses a nuclear localization signal in the N-terminal non-catalytic region (residues 38–43) and a kinase domain (residues 49–309) [38]. The N-terminal and C-terminal non-catalytic regions of LKB1 are not related to any other proteins and possess no identifiable functional domains [1,2]. LKB1 has now been classified as a member of the calcium/calmodulin regulated kinase-like family, which is part of the Ca<sup>2+</sup>/calmodulin kinase group ([http:// www.kinase.com](http://www.kinase.com)).

Human LKB1 shows strong homology to the cytoplasmic serine/threonine kinases of several organisms, including *Xenopus laevis* egg and embryonic kinase 1 (XEEK1), mouse LKB1, *Caenorhabditis elegans* partitioning defective gene 4 (Par-4), and *Drosophila* LKB1 [39–42].

There is a 92.5% similarity between mouse and human *LKB1* genes, and 97.5% in their core kinase domain. Similarly, there is 84.5% and 96.2% similarity between the core kinase domains of LKB1 of mouse and xenopus, respectively [43,44].

LKB1 is expressed ubiquitously in adult and fetal tissues, especially in pancreas, liver, testes, and skeletal muscle [45–49]. LKB1 signaling is regulated through two main mechanisms: phosphorylation and subcellular localization. LKB1 can be phosphorylated on at least eight residues [42,50–56], and its phosphorylation is thought to play a key role in cell cycle arrest, tumor suppression, and cell polarity [38].

*In vivo*, LKB1 forms a heterotrimeric complex with two proteins termed STE20-related adaptor (STRAD) and mouse protein 25 (MO25), which play a crucial role in regulating LKB1 protein stability, kinase activity, and cellular localization [54,57,58]. Although STRAD possesses STE20-like kinase domain, it has been classified as a pseudokinase because it lacks key residues present in other kinases that are required for catalysis [57]. The binding of STRAD to LKB1 substantially activates the autophosphorylation of LKB1 and hence its ability to phosphorylate downstream substrates. STRAD could also direct the subcellular localization of LKB1 by anchoring it in the cytoplasm [57]. MO25 is a scaffolding protein of 40 kDa that stabilizes the binding of STRAD to LKB1, stimulating the catalytic activity of LKB1 ~10-fold [54]. The crystal structure of full-length MO25 $\alpha$  in complex with a peptide encompassing the C-terminus of STRAD $\alpha$  reveals that MO25 $\alpha$  binds

specifically to a conserved Trp-Glu-Phe sequence at the STRAD $\alpha$  C terminus, greatly enhancing the association of STRAD $\alpha$  with LKB1 by generating additional binding site(s) on STRAD $\alpha$  [54,59]. Dorfman *et al.* [60] have recently provided a model of the mechanism that determined the nucleocytoplasmic transport of the LKB1 protein kinase. It is demonstrated that STRAD $\alpha$  is indispensable for the cytoplasmic localization of LKB1 as there is no nuclear export signal in LKB1. In the nucleus, STRAD $\alpha$  acts as an adaptor capable of conjoining LKB1 to exportins CRM1 and exportin 7, facilitating nuclear export of LKB1. Also, in the cytoplasm, STRAD $\alpha$  serves as a competitor to importin- $\alpha/\beta$  for binding to LKB1, inhibiting reimport of LKB1. There is no evidence of MO25 directly facilitating the nucleocytoplasmic shuttling of LKB1-STRAD $\alpha$  complex. The function of MO25 seems to be limited to enhance the affinity of STRAD $\alpha$  for LKB1 [60]. It has been demonstrated that the activities of many signaling proteins associated with cancer are regulated by nucleocytoplasmic shuttling [61,62]. It is plausible that translocation between various cellular compartments allows for an additional level of spatial regulation of the LKB1 protein kinase activity.

### Role of *LKB1* in Regulating Cell Polarity

No matter for constructing the cellular and whole body architecture or for maintaining cellular and tissue function, the importance of establishing and maintaining proper cell polarity can never be over emphasized in multicellular organisms. Loss of cell polarity strongly correlates with more aggressive and invasive growth of malignant cells in human cancers [63]. The role of LKB1 in regulating cell polarity was first discovered by the Kemphues *et al.* [64] through analyzing *C. elegans* mutants. Mutations in the partitioning defective gene 4 (*par-4*), the counterpart of mammalian *LKB1* in *C. elegans*, have been demonstrated to disrupt the asymmetries established normally during the first cell cycles of embryogenesis and several other aspects of cell polarity [65,66]. To identify additional genes involved in anterior-posterior (A-P) axis formation in *Drosophila*, Martin and St Johnston [42] carried out a genetic screen in germline clones for mutants that disrupt the localization of Staufen. During this screen, they found a novel gene, named *Drosophila LKB1*, encodes a serine/threonine kinase with strong homology to *C. elegans* Par-4 and to the human tumor-suppressor LKB1. It is demonstrated that *Drosophila LKB1* is critical for the early

A-P polarity of the oocyte, and for the formation of the embryonic A-P axis. Mutation of *Drosophila LKB1* displays disrupted polarity and disrupted A-P patterning [42].

Baas *et al.* [34] have provided the most convincing evidence that LKB1 also regulates cell polarization in a mammalian system. In an STRAD-inducible system, activation of LKB1 has been found to reorganize the actin cytoskeleton and to relocalize some junctional proteins, inducing the formation of a brush border and gap junctions even in the absence of cell-cell contacts [34]. Consistently, more recent studies have also suggested that mammalian LKB1 helps to establish cell polarity [51,52]. Barnes *et al.* [51] disrupted expression of LKB1 in the murine neocortex using conditional knockout approach to demonstrate that LKB1 does indeed function during axon specification, specifically in neuronal polarization in the mammalian cortex. In complementary work, Shelly *et al.* [52] confirmed that down-regulation of either LKB1 or STRAD by siRNAs prevented axon differentiation, and overexpression of these proteins led to multiple axon formation. These studies, although predominantly in neurons, nonetheless demonstrate LKB1's key role in cell polarity in mammals.

At this stage, the question mainly arises as to the nature of the LKB1's effects involved in the modulation of cell polarity. As a master kinase, LKB1 can activate several downstream kinases; among those, Par-1/MARK plays a key role in regulating cell polarity in numerous cell types and organisms [28,29,32]. The Par-1, a serine/threonine protein kinase, was originally identified in a study published in 1988 designed to identify regulators of early embryonic polarity in *C. elegans* [64]. Further studies done by many groups have demonstrated that Par-1 is a evolutionarily conserved proteins and it regulates cell polarity not only in *C. elegans* but also in *Drosophila*, *Xenopus*, and mammals [34,42,66-68]. In humans, there are four closely related Par-1-like isoforms: MARK1, MARK2, MARK3, and MARK4 [69]. It is demonstrated that these MARK isoforms are phosphorylated and activated by LKB1 at Thr residue on their T-loop [27]. Taken together, these results implicated that LKB1 serves as a master regulator of cellular polarity, at least in part, by activating the Par-1/MARK. However, it has been observed that overexpression of LKB1 can partially rescue the polarity defects in Par-1 mutants [42]. Thus, it is possible that other downstream kinases are also involved in the LKB1-dependent cell polarity regulation.

The brain-specific kinase 1 (BRSK1, also known as SAD-A) and BRSK2 (SAD-B) that are activated by LKB1 are mainly expressed in the brain and at low levels in the testis [70]. SAD-A (BRSK1) was originally identified as a novel serine/threonine kinase in *C. elegans* in 2001 [71]. Crump *et al.* demonstrated that SAD-A (BRSK1) controlled several aspects of presynaptic differentiation, such as presynaptic vesicle clustering and axon termination. SAD-A loss-of-function mutants in *C. elegans* display polarity defects in neurons [71]. Similarly, mice lacking both the SAD-A and the SAD-B kinases were reported to die at birth because of failure of their neurons to polarize and form axons and dendrites, showing that these kinases are required for neuronal polarity in mammals [70]. More recently, Hung *et al.* have further confirmed that SAD-A interact directly with Neurabin, a scaffolding protein, regulating neuronal polarity in *C. elegans*. They have proposed that Neurabin facilitates SAD-A to phosphorylate substrates that are specific for synapse formation during neuronal polarization [72]. Collectively, these results suggest that the BRSK (SAD) kinases, activated by LKB1, may play a specific role in regulating polarization of neuronal cells.

For a long time, there was a hypothesis that LKB1 signaling mediated by AMPK has been limited to its role in controlling cellular energy metabolism [30]. Quite recently, however, two independent groups almost simultaneously demonstrated that AMPK also plays a role in maintaining normal cellular architecture and cell polarity [73,74]. Lee *et al.* [73] generated the first *ampk*-null mutants in animal model, surprisingly, to discover that AMPK is a critical downstream mediator of LKB1 in controlling mitotic cell division and epithelial polarity. It has been observed that, when completely deprived of the activity of AMPK, flies could not survive before the mid-pupal stage with severe abnormalities in cell polarity and mitosis. However, the phenotypes could be rescued by transgenic expression of wild-type AMPK [73]. Consistently, a second study showed that AMPK are required to maintain epithelial polarity and cell proliferation under energetic starvation conditions in *Drosophila melanogaster* [74].

By strongly reducing the availability of sugar in the culture medium, *ampk* $\alpha$  mutant epithelial cells displayed a completely disrupted apical–basal polarity, accompanied by a sharply reduced amount of basal stress fibers and an increased amount of apical F-actin in *ampk* $\alpha$  mutant follicle cells [74].

Additionally, Lee *et al.* have also provided a plausible answer to the question of how AMPK controls cell

polarity. They have proposed that the regulatory light chain of non-muscle myosin II (MRLC, also known as MLC2) is the physiological substrate of AMPK. It has been observed that the expression of a constitutively active MRLC, whose regulatory phosphorylation site was mutated into phosphomimetic glutamates, not only can rescue the epithelial polarity defects caused by the loss of AMPK but also can restore the epithelial polarity defects of *lkb1*-null wing imaginal discs [73]. These results provide a potential mechanism for LKB1 signals through AMPK to coordinate epithelial polarity and proliferation with cellular energy status, and this might underlie the tumor suppressor function of LKB1.

More recently, a novel LKB1–Cdc42–p21-activated kinase (PAK) pathway has been proposed to regulate cell polarity in non-small cell lung cancer [75]. It has been demonstrated that Cdc42, a small GTPase of the Rho family, plays a central role in establishing cell polarity in all eukaryotic cells extending from yeast to mammals. In its active GTP-bound form, Cdc42 can potentially interact with multiple targets involved in cell polarity establishment [76]. One of the best-characterized targets of Cdc42 is the PAK, the key role of which is to regulate cytoskeletal dynamics and cell motility, through phosphorylating MLC [77]. Zhang *et al.* [75] have recently observed that when cell motility and polarity are stimulated, LKB1 co-localizes with cdc42, PAK at the cellular leading edge, which leads to increased PAK phosphorylation and promotion of downstream cell polarity events. Furthermore, the phosphorylation of PAK declined owing to reduced cdc42 activation in LKB1-depleted cell lines, verifying the value of the LKB1–cdc42–PAK pathway in cell polarity again [75].

## Role of *LKB1* in Regulating Metabolism

The AMPK is an evolutionally conserved Ser/Thr kinase that functions as a key regulator of cellular energy metabolism [30,35]. AMPK is a heterotrimeric complex comprising a catalytic AMPK $\alpha$  subunit and regulatory AMPK $\beta$  and AMPK $\gamma$  subunits. The  $\gamma$ -subunit has 4-cystathionine- $\beta$ -synthase domains, which are required for the binding of AMP or ATP, increasing or decreasing AMPK activity, respectively [35]. Binding of AMP to the  $\gamma$ -subunit is thought to stimulate phosphorylation of Thr172 in the T-loop of AMPK $\alpha$  catalytic subunit as well as to maintain AMPK activity by preventing dephosphorylation of the Thr172 site [35]. There had been many attempts to identify the upstream protein kinase(s) that phosphorylate Thr172; however, no obvious

candidates were found prior to 2003 [78]. In 2003, for the first time, Hawley *et al.* provided strong evidence that LKB1 is the long sought-after upstream kinase of AMPK. It was observed that AMPK could not be activated by the AMPK-activating drugs AICA riboside (AICAR) and phenformin in HeLa cells (without expression of LKB1); however, the activation could be restored by stably expressing wild-type LKB1 [78]. Consistently, a subsequent study by Shaw *et al.* proposed biochemical and genetic evidence that LKB1 is a major activator of AMPK in several mammalian cell types. They have also demonstrated that LKB1 directly phosphorylates Thr-172 of AMPK $\alpha$  *in vitro*, which results in its activation. In addition, it has been shown that AMPK is strikingly desensitized to a variety of stimuli in LKB1-deficient murine embryonic fibroblasts, which can be restored by reintroduction of wild-type LKB1 [79].

As its name suggests, AMPK is allosterically activated by 5'-AMP, an effect antagonized by high concentrations of ATP. Because of the reaction catalyzed by adenylate kinase ( $2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$ ), the AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio, making the former ratio (essentially the parameter to which AMPK responds) a sensitive indicator of reduced cellular energy status [79,80]. Consequently, any cellular or metabolic stress (such as glucose deprivation, hypoxia, oxidative stress, hyperosmotic stress, tissue ischemia, and muscle contraction/exercise) that either inhibits ATP synthesis or accelerates ATP consumption will cause AMPK activation [81]. Once activated, AMPK 'switches off' many anabolic pathways and 'switches on' catabolic pathways that serve to restore intracellular ATP level [82].

Early studies provided evidence that AMPK mediates glucose transport in skeletal muscle. AICAR-induced activation of AMPK causes insulin-independent increases in skeletal muscle glucose transport, suggesting that the activation of AMPK can lead to the activation of glucose transport in skeletal muscle [83]. AMPK has also been suggested to be a critical regulator of fatty acid oxidation by phosphorylating and inactivating acetyl CoA carboxylase, which results in decreased production of the carnitine palmitoyltransferase I (CPT1) inhibitor, malonyl-CoA. CPT1 promotes fatty acid transport into mitochondria for subsequent oxidation [84,85]. Furthermore, Koh *et al.* [86] have found that muscle-specific *LKB1* knockout mice have elevated intramuscular triglycerides. Using a similar muscle-specific *LKB1* knockout mouse model, Thompson *et al.* [87] have shown impaired AICAR-induced fatty acid oxidation.

Taken together, LKB1-AMPK pathway plays a critical role in regulating glycolysis and fatty acid oxidation to maintain cellular energy levels immediately through short-term effect on phosphorylation of regulatory proteins.

In addition, LKB1-AMPK pathway exerts more long-lasting effects to restore intracellular energy level by targeting both transcription and translation to inhibit cell growth and proliferation [88-90]. The inhibition of translation through cross-talk with the TSC1/TSC2-mTOR signaling pathway is a typical example [91,92]. Recent studies from several laboratories have demonstrated that LKB1 is necessary for mammalian target of rapamycin (mTOR) repression under low ATP conditions in an AMPK- and TSC2-dependent manner [91,92]. When activated by LKB1 under energy stresses, AMPK has been shown to phosphorylate and activate TSC2, which then suppresses mTOR signaling toward the key translational regulators 4E-BP1 and S6K1 [91]. Consistent with these observations, LKB1-deficient mouse embryonic fibroblasts and gastrointestinal polyps derived from *LKB1* mutant mice have been found to exhibit elevated levels of S6K1 activity and phosphorylation of 4E-BP1, indicating an aberrantly elevated mTOR signaling. Furthermore, reintroduction of wild-type LKB1 into HeLa cells restores AICAR-dependent inhibition of S6K1 and 4E-BP1 phosphorylation in response to ATP depletion, indicating that LKB1-dependent mTOR regulation is conserved in different cell types [92]. In short, these studies provided an indication that LKB1 exerts its long-lasting effects in regulating metabolism partly through its ability to regulate the mTOR pathway.

However, it seems likely that additional LKB1 effectors intervening in metabolism should also be characterized. As is discussed above, the Ser/Thr protein kinase Par-1b/MARK2/Emk is part of a larger subfamily of kinases that includes 13 members, most of which appear to be regulated by LKB1 [27-29]. The Par-1b/MARK2 protein kinase is conserved from yeast to human and has been shown in multiple organisms and cell types to be critical for regulation of cellular polarity [34,42,66,67]. Surprisingly, recent studies by Hurov *et al.* [36] have suggested that the polarity kinase Par-1b/MARK2/Emk is a novel regulator glucose metabolism *in vivo*. It is the first study that this kinase is implicated in metabolic functions akin to those previously defined for AMPK. A series of remarkable metabolic disorders have been observed in Par-1b null mice, including decreased adiposity, hypermetabolism, insulin hypersensitivity,

resistance to diet-induced obesity, and enhanced glucose uptake into white and brown adipose tissue [36]. Taken together, the finding that Par-1b plays a critical role in regulating glucose metabolism and energy balance proposes the exciting possibility that this kinase may be a valuable drug target for the treatment of type 2 diabetes and obesity.

## **LKB1 in the Control of Cell Proliferation and Apoptosis**

The polyp formation in PJS could be due to the increased cell proliferation or decreased apoptosis, and/or the involvement of both pathways [93]. Several studies have indicated that overexpression of wild type LKB1 in various cancer cell lines, such as HeLa and G361, which do not express endogenous LKB1, suppressed the proliferation of these cells by inducing a G1 cell cycle arrest [94–96]. However, such growth arrest has not been observed with most of the catalytically inactive LKB1 mutants, including some of those isolated from PJS patients [94]. Thus, it is assumed that catalytic activity of LKB1 is required to block cell division and total loss of LKB1 expression seems to be a precondition of polyp formation [97].

It has been found that LKB1-mediated G1 arrest is overcome by co-expression of cyclin D1 or cyclin E, which suggests that LKB1 signaling leads to a decrease in the activity of endogenous G1 cyclin-dependent kinase (CDK)–cyclin complexes. Further studies have demonstrated that forced LKB1 expression in G361 cells, a melanoma cell line that lacks endogenous LKB1, enhances the expression of the CDK inhibitor p21/WAF1 through activating its transcription from promoter. Moreover, both LKB1-mediated induction of p21 and LKB1-mediated growth arrest are proved to be p53-dependent [98]. Zeng *et al.* [99] have confirmed and extended previous observations by demonstrating that LKB1 binds to and stabilizes p53 in the nucleus and phosphorylates p53 at Ser15 and Ser392, directly or indirectly, which are required for p53-dependent cell cycle G1 arrest. However, a p53-independent mode of p21 up-regulation by LKB1 has also been observed in p53-dull HeLaS3 cells, in which LKB1 bypasses the need of p53 and forms a complex with LMO4, GATA-6, and Ldb1 to induce p21 expression [100]. Remarkably, Scott *et al.* [101] have recently confirmed that LKB1 arrests cells in G1 phase of the cell cycle

independent of either p21 or p53, whereas the combination of LKB1, p21, and p53 moderately improved LKB1-mediated G1 arrest. In addition, LKB1 has also been found to bind to and regulate brahma-related gene 1 (Brg1), which is necessary for Rb-induced cell cycle arrest in both G1 and S phases, suggests that LKB1 might also function in the Brg1/Rb signaling pathway to induce growth arrest [37].

On the other hand, studies on the absence of apoptosis in PJS polyps reveal that LKB1 is an inducer of apoptosis *in vivo* [102–105]. Karuman *et al.* have demonstrated first that LKB1 can induce apoptosis in epithelial cells and that the kinase activity of LKB1 is required for the induction of apoptosis. Furthermore, they have proposed that p53 and LKB1 can physically interact and that the presence of functional p53 is critical in mediating LKB1-induced apoptosis, as overexpression of LKB1 induced apoptosis mainly in cells with functional p53. However, it has also been found that p53<sup>-/-</sup> mice do not exhibit a defect in apoptosis of stem cells where LKB1 is clearly activated in dying cells, which might indicate the existence of a LKB1-mediated p53-independent apoptosis pathway [102]. Previous studies have suggested that the c-Jun N-terminal kinase (JNK) is a member of MAPK family, activation of which is required for the release of cytochrome *c* from the mitochondria and the subsequent activation of the caspase-mediated apoptosis [103]. Using the *Drosophila* model system, Lee *et al.* [104] have recently discovered a genetic connection between LKB1 and the JNK pathway and have demonstrated that the JNK pathway acts as a downstream effector of LKB1 by mediating the LKB1-induced apoptosis. LKB1 activates JNK via the Hep-dependent pathway, and this activation is one of the crucial functions of endogenous LKB1 during embryonic neuronal development in *Drosophila*, whereas blockage of the JNK signaling pathway strongly suppresses the LKB1-induced apoptosis. Surprisingly, the LKB1-induced apoptosis has been found to be caspase-dependent but p53-independent in the *Drosophila* model system, as it is not suppressed at all by the dominant-negative p53, whereas completely suppressed by *Drosophila* inhibitor of apoptosis [104]. Furthermore, it has also been observed that LKB1 is dispensable for p53-dependent apoptosis in the DNA damage-induced apoptotic signaling [105]. Therefore, it is interesting to find that LKB1 and p53 keep each other at an arm's length, as they work either in collaboration or independently to mediate cell apoptosis responding to different cell types and cell conditions.

Remarkably, recent studies have suggested that LKB1 acts as either an inducer or a suppressor of apoptosis depending on the severity of energy stress and cell types [79,106]. Contrary to the observations that LKB1 can mediate apoptosis under normal energy conditions, Shaw *et al.* have found that LKB1 is essential to protect cells from apoptosis under energy stress, whereas LKB1-deficient cells are hypersensitive to apoptosis in response to elevated intracellular AMP. Moreover, LKB1-deficient cells are also sensitized to death induced by the AMP analog AICAR. Therefore, it is suggested that LKB1-AMPK signaling plays a role in protecting cells from apoptosis in response to agents that increase the cellular AMP/ATP ratio [79]. Consistent with this view, in more recent studies, Mukherjee *et al.* [106] have demonstrated that energy stress induced by glucose withdrawal or addition of 2-deoxyglucose causes more ATP depletion, AMPK phosphorylation, and apoptosis in LKB1-deficient mouse astrocytoma cells than in syngeneic normal astrocytes. Taken together, it is hypothesized that active LKB1-AMPK signaling offers a protective effect by allowing the cell time to attempt to reverse the aberrantly high ratio of AMP/ATP. If unable to reverse this ratio, however, the cell eventually will undergo apoptosis [79,106].

## Conclusions and Perspectives

Ten years have now passed since *LKB1* was identified as the causative gene of PJS [1,2]; remarkable progress has been made in the structural and functional analysis of LKB1 in human and model organisms. LKB1 is now known to be critically important not only in PJS, but also in more common disorders, including diabetes and cancers. Thus far, LKB1 has been found to play a role in cell polarity, energy metabolism, apoptosis, cell cycle arrest, and cell proliferation, all of which may require the tumor suppressor function of this kinase and/or its catalytic activity. The findings outlined in this review also provide medical benefits in the form of rational treatment strategies to individuals suffering from PJS and the more common disorders related to LKB1.

As discussed above, LKB1 does not represent an ideal drug target itself because drugs that inhibited LKB1 might be expected to induce tumors. However, the inhibition of LKB1 or AMPK pathway might also be supposed to make cancer cells more sensitive to apoptosis and thus make cells more resistant to cell transformation by conventional oncogenes through reducing the ability

of tumor cells to tolerate and survive low energy conditions, such as hypoxic situations found in tumors or following chemotherapy [38]. How on earth does the LKB1-AMPK pathway act to suppress tumorigenesis or to rescue cancer cells from metabolic collapse? A new breakthrough from the Alessi laboratory has given a conclusive answer that the LKB1-AMPK pathway plays a vital role in suppressing tumorigenesis in PTEN (phosphatase and tensin homolog deleted on chromosome 10)-deficient mice [107]. It has been observed that AMPK-activating drugs (metformin, phenformin, and A769662), traditionally used to counter the metabolic changes observed in diabetes, effectively restrain cancer cell growth in *PTEN*<sup>+/-</sup> mice. Furthermore, they have also proposed that it is inadvisable to pharmacologically inhibit LKB1 and/or AMPK, at least for the treatment of cancers in which the mTORC1 pathway is activated. Collectively, these results have demonstrated that the activation of AMPK by AMPK-activating drugs, such as clinically approved metformin and phenformin, is likely to be of significant therapeutic value in a wide spectrum of human tumors [107]. In future work, it would be interesting to further explore the anticancer effects of metformin and the potential medical benefits offered by targeting the AMPK/mTOR signaling pathway. Further investigation is also required to provide the details of LKB1 protein structure and its enzymatic kinetics to define better how LKB1 activity modulates its downstream substrates. To obtain high-level expression of LKB1, however, seems to be a major obstacle to study its crystal structure and function *in vitro*. Liu *et al.* [108] have recently established a high-level expression system of active human LKB1 in *E. coli* which may be helpful to studies in this field.

As mentioned above, loss-of-function mutations in LKB1 are particularly common in human lung cancers [6]. In human lung cancer, loss of LKB1 is frequently associated with gain-of-function mutations in Ras. Other challenges for future research will be to understand why mutations of *LKB1* are so prevalent in lung cancer and the role that *Ras* and/or carcinogens play in promoting transformation of LKB1-deficient lung cells.

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