The LKB1–AMPK pathway: metabolism and growth control in tumour suppression

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Abstract | In the past decade, studies of the human tumour suppressor LKB1 have uncovered a novel signalling pathway that links cell metabolism to growth control and cell polarity. *LKB1* encodes a serine–threonine kinase that directly phosphorylates and activates AMPK, a central metabolic sensor. AMPK regulates lipid, cholesterol and glucose metabolism in specialized metabolic tissues, such as liver, muscle and adipose tissue. This function has made AMPK a key therapeutic target in patients with diabetes. The connection of AMPK with several tumour suppressors suggests that therapeutic manipulation of this pathway using established diabetes drugs warrants further investigation in patients with cancer.

Peutz–Jeghers syndrome
A disorder that is characterized by the development of gastrointestinal hamartomas and an increased predisposition to many other malignancies, including those arising in colon, breast, ovarian, pancreatic and lung tissues.

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A fundamental requirement of all cells is that they couple nutrient availability to the signals that emanate from growth factors to drive proliferation only when nutrients are in sufficient abundance to guarantee successful cell division. Although a connection between cellular metabolism and tumorigenesis was first proposed 100 years ago by Otto Warburg, the molecular mechanisms that interconnect the signalling pathways controlling metabolism and cell growth have only begun to be decoded in the past decade, making this an active area of investigation in cancer research. One of the newly uncovered links directly connecting cell metabolism and cancer came from the discovery that the serine–threonine kinase liver kinase B1 (LKB1; also known as STK11), a known tumour suppressor, was the key upstream activator of AMP-activated protein kinase (AMPK)[1–4]. AMPK is a central metabolic switch found in all eukaryotes that governs glucose and lipid metabolism in response to alterations in nutrients and intracellular energy levels.

LKB1 was identified originally as the tumour suppressor gene on human chromosome 19p13 responsible for the inherited cancer disorder Peutz–Jeghers syndrome (PJS)[5]. Importantly, *LKB1* is also one of the most commonly mutated genes in sporadic human lung cancer, particularly in multiple subtypes of non-small cell lung carcinoma (NSCLC)[6], in which at least 15–35% of cases have this lesion[7]. *LKB1* was also recently found to be somatically mutated in 20% of cervical carcinomas[8], making it the first recurrent genetic alteration to be identified in this type of cancer, that cooperates with human papilloma virus infection to promote disease progression.Together, LKB1 and AMPK control cell growth in response to environmental nutrient changes, which — as we discuss in this Review — identifies new targets and drugs for cancer therapy, including several existing diabetes therapeutics that are known to potently activate AMPK. In addition to controlling cell growth and metabolism, both LKB1 and AMPK have conserved roles in cell polarity, the disruption of which is also implicated in carcinogenesis. As LKB1 is one of the few serine–threonine kinases that is known to be inactivated through mutation during carcinogenesis, a crucial early step was to identify its substrates.

LKB1 is a master kinase

The search for substrates of LKB1 that mediate its tumour suppressor function led to the identification of AMPK as a direct LKB1 substrate[1–4]. AMPK is a heterotrimer composed of a catalytic (AMPKα) subunit and two regulatory (AMKPβ and AMPKγ) subunits (FIG. 1). AMPK is activated when intracellular levels of ATP decline and intracellular levels of AMP increase, such as during nutrient deprivation or hypoxia. Biochemical and genetic analyses in worms, flies and mice have shown that LKB1 is the major kinase that phosphorylates the AMPKα activation loop in conditions of energy stress[9].

LKB1 also phosphorylates and activates 12 kinases that are closely related to AMPK[10,11] (FIG. 2). Of the 14 kinases, most current data suggest that only AMPKα1 and AMPKα2 are activated in low ATP conditions, probably because only they interact with AMPKγ[12]. Interestingly, 4 of these 14 kinases are members of the

At a glance

- The serine–threonine liver kinase B1 (LKB1) is inactivated in Peutz–Jeghers syndrome and a large percentage of sporadic non-small cell lung carcinomas and cervical carcinomas.
- LKB1 acts a master upstream kinase, directly phosphorylating and activating AMP-activated protein kinase (AMPK) and a family of 12 related kinases that have crucial roles in cell growth, metabolism and polarity.
- The LKB1–AMPK pathway serves as a metabolic checkpoint in the cell, arresting cell growth in conditions of low intracellular ATP levels, such as in low nutrient conditions.
- One of the central mitogenic pathways that is suppressed by LKB1 and AMPK signalling is the mTOR complex 1 pathway, which is inhibited through AMPK phosphorylation of tuberous sclerosis complex 2 and regulatory associated protein of mTOR (raptor).
- Overnutrition and hyperglycaemia can suppress LKB1–AMPK signalling, which might contribute to an increased cancer risk in patients who are obese or diabetic. Conversely, activation of LKB1–AMPK signalling might contribute to the suppression of cancer risk that is associated with exercise and caloric restriction. Will AMPK-activating drugs, including existing diabetes therapeutics, find clinical usefulness as anticancer agents?

microtubule-associated protein (MAP) and microtubule affinity-regulating kinase (MARK; also known as Par-1) family, which are mammalian homologues of the *Caenorhabditis elegans* PAR-1 kinase that is required for early embryonic partitioning and polarity. *Par-4* encodes the *C. elegans* orthologue of *LKB1* (REF. 13). The ability of LKB1 or its orthologues to act as master upstream kinases that activate AMPK, MARK and several additional AMPK-related kinases seems to be widely conserved across eukaryotes.

From tissue-specific knockouts of *Lkb1* in mice (TABLE 1), it seems that LKB1 dictates most of the AMPK activation in all tissues that have been examined so far with the exception of some hypothalamic neurons[14], T cells[15] and endothelial cells[16], in which calcium and calmodulin-dependent protein kinase kinase 2 (CAMKK2) seems to play a key part in AMPK activation, although only in response to changes in the concentration of calcium[17–19]. LKB1 therefore uniquely mediates the prolonged and adaptive activation of AMPK following energy stress, which allows it to serve as a metabolic checkpoint.

A LKB1–AMPK–mTORC1 checkpoint

Prior to its identification as a substrate for LKB1, AMPK was known to regulate lipid, cholesterol and glucose metabolism in specialized metabolic tissues, such as liver, muscle and adipose tissue[20]. In the past 5 years, work from several laboratories has shown that one of the major growth regulatory pathways controlled by LKB1–AMPK is the mTOR pathway. mTOR is a central integrator of nutrient and growth factor inputs that controls cell growth in all eukaryotes and is deregulated in most human cancers[21].

mTOR is found in two biochemically and functionally discrete signalling complexes[22]. mTOR complex 1 (mTORC1) includes regulatory-associated protein of mTOR (raptor), which acts as a scaffold to recruit downstream substrates, such as eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and ribosomal S6 kinase (S6K1), which contribute to mTORC1-dependent regulation of protein translation[23]. mTORC1 controls the translation of many cell growth regulators, including cyclin D1, hypoxia inducible factor 1a (HIF1α), and MYC, which in turn promote processes that include cell cycle progression, cell growth and angiogenesis, all of which can become deregulated during tumorigenesis[21]. mTORC1 is nutrient sensitive and acutely inhibited by rapamycin, although recent studies show that rapamycin does not fully suppress mTORC1 activity in many cell types[24–26]. By contrast, mTORC2 contains the rapamycin-insensitive companion of mTOR (rictor) subunit and is neither sensitive to nutrients nor acutely inhibited by rapamycin[21].

Cancer genetics and *Drosophila melanogaster* genetics led to the discovery of upstream components of mTORC1, including the tuberous sclerosis complex 2 (TSC2) tumour suppressor and its obligate partner TSC1 (REF. 27). TSC2 inhibits mTORC1 indirectly through the regulation of the small GTPase Ras homologue enriched in brain (RHEB), such that loss of TSC1 or TSC2 leads to hyperactivation of mTORC1[28]. When the levels of ATP, glucose or oxygen are low, AMPK directly phosphorylates TSC2 on conserved serine sites[29–32] and primes serine residues close to these conserved sites for subsequent phosphorylation by glycogen synthase kinase 3 (GSK3)[33]. Wnt signalling inhibits the phosphorylation of TSC2 by GSK3, making TSC2 activity a biochemical coincidence detector for the activation states of AMPK and GSK3. The activation states of these two kinases dictate the amount of downstream mTORC1 signalling that occurs.

Although TSC2 is clearly a central receiver of inputs that regulate mTORC1, cells lacking TSC2 still partially suppress mTORC1 following AMPK activation[34,35]. In agreement with these data, raptor has been identified as a direct substrate of AMPK *in vivo*. Phosphorylation of two conserved serines in raptor by AMPK induced the binding of raptor to 14-3-3 and resulted in the suppression of mTORC1 kinase activity[35]. Phosphorylation of raptor was shown to be required for downregulation of mTOR and efficient G2/M cell cycle arrest following AMPK activation[35]. Taken together, the current data indicate that energy stress results in LKB1-dependent activation of AMPK, which directly phosphorylates both TSC2 and raptor to inhibit mTORC1 activity by a dual mechanism, although it remains possible that additional substrates of AMPK contribute to the regulation of mTOR (FIG. 3). Importantly, mTORC1 is currently the only signalling pathway downstream of LKB1 that has been shown to be deregulated in tumours that arise in humans and mouse models of both PJS[31,36] and NSCLC[7,37].

LKB1–AMPK control of other growth regulators

LKB1 has also been reported to regulate key cancer-related pathways other than mTORC1. Most notably, several connections have been made between LKB1, AMPK and the tumour suppressor p53. Before any direct substrates for LKB1 were identified, LKB1 reconstitution into LKB1-deficient tumour cells was reported to stimulate

Tuberous sclerosis complex
A familial tumour syndrome that is induced through mutation of the mTOR complex 1 regulators TSC1 and TSC2.

p53 activity and increase the levels of *CDKN1A* mRNA, which encodes the cyclin-dependent kinase inhibitor p21 (REFS 38,39). In addition, AMPK has been shown to modulate p53-dependent apoptosis[40] and directly phosphorylate p53 on serine 15 (REF. 41), which is an established site for phosphorylation by the ataxia-telangiectasia mutated (ATM), ataxia–telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) DNA-damage response kinases[42]. Several studies have indicated that AMPK is also activated downstream of p53 (REF. 43), and this led to the discovery of sestrin 1 (*SESN1*) and sestrin 2 (*SESN2*) — p53 target genes that inhibit mTOR signalling[44]. Overexpression of *Sesn1* or *Sesn2* leads to increased AMPK activation and suppression of mTORC1 signalling, whereas mice that lack *Sesn2* fail to downregulate mTORC1 following exposure to carcinogens. The molecular mechanism by which sestrins activate AMPK in this context remains to be fully elucidated. In addition to the sestrins, *PRKAB1*, which encodes the AMPK β1 regulatory subunit, is a p53-responsive gene, suggesting the existence of another mechanism through which p53 can inhibit mTOR[45]. Importantly, AMPK has been shown to phosphorylate a conserved serine in forkhead box O3a (FOXO3a), a transcription factor that is targeted by PI3K–Akt signalling and plays key parts in cell survival and metabolism[46]. Notably, the best-mapped AMPK site in FOXO3a matches the consensus for 14-3-3 binding, which is also the case for the best-mapped AMPK site in TSC2 (FIG. 2). The parallel regulation of FOXO3a and mTOR signalling by AMPK and Akt signalling suggests that further study is warranted to investigate the functional overlap between these central pathways that control both cell growth and metabolism.

AMPK has also been reported to phosphorylate threonine 198 of the cyclin-dependent kinase inhibitor p27 (REFS 47,48). However, T198 has also been reported to be phosphorylated by Rsk, Akt and Pim kinases, which promote cell growth. Why these pro-growth and anti-growth signals would both target the same phosphorylation site has yet to be established. Several additional AMPK substrates have been suggested to have a role in growth regulation[49,50]. However, future studies with rigorously validated phospho-specific antibodies for each phosphorylation site and careful analysis of the early time points that follow acute energy stress in wild-type or AMPK-deficient cells should help to assign which of these candidate targets are bona fide AMPK substrates *in vivo*.

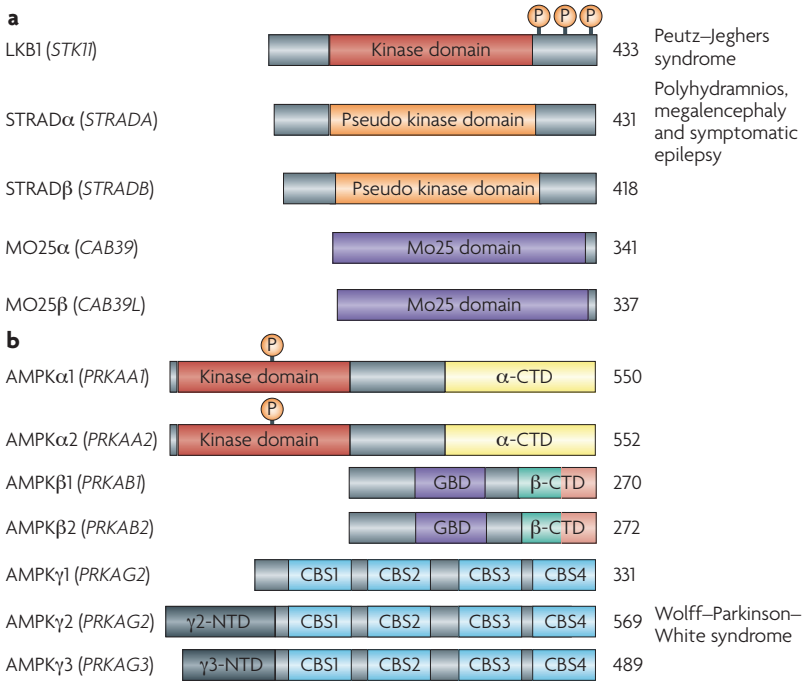


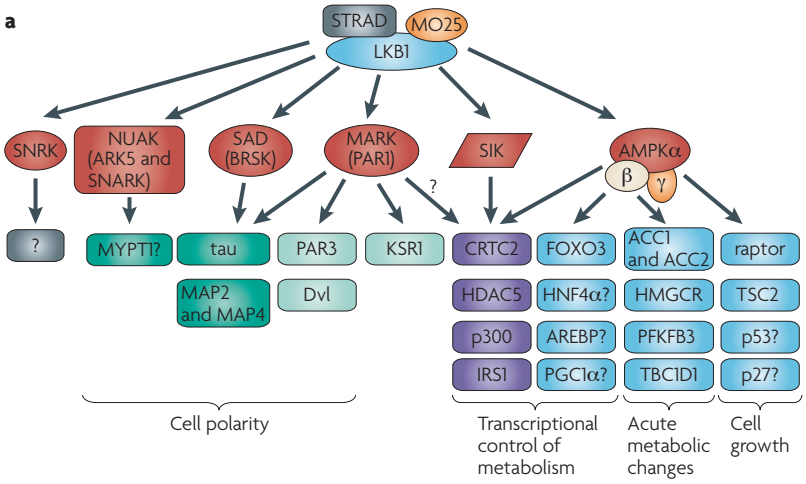
Figure 1 | **Proteins in the liver kinase B1 and AMP-activated protein kinase complexes.** Both liver kinase B1 (LKB1) and AMP-activated protein kinase (AMPK) exist in heterotrimeric protein complexes. Inactivating mutations in *LKB1* underlie the inherited cancer disorder Peutz–Jeghers syndrome. In addition to deletions or frameshifts, several missense mutations have been found in *LKB1* and most cluster in the kinase domain, resulting in loss of kinase activity. A small number of mutations lie outside the kinase domain and some of these have been shown to result in decreased kinase activity owing to disruption of protein–protein interactions between LKB1 and its regulatory subunits STE20-related adaptor (STRAD) and MO25 (also known as CAB39), which seem to be necessary for its kinase activity[186]. Together, the genetic evidence indicates that the tumour suppressor function of LKB1 requires its kinase activity. Although there is a single *LKB1* gene in mammals, two STRAD and two MO25 family members exist, and mutations in STRADα underlie the development of an inherited epileptic disorder[187]. There are two known splice forms of LKB1, which differ in the most carboxy-terminal amino acids[188,189]. Evidence indicates that STRAD proteins also undergo extensive alternative splicing[190]. Similarly to LKB1, AMPK is composed of a catalytic subunit (α) and two regulatory subunits. The β-subunits contain a conserved glycogen-binding domain that also modulates AMPK activity[191]. The γ-subunits contain a series of tandem repeats of crystathionine-β-synthase (CBS) domains to which molecules of AMP bind, as shown in recent X-ray crystallography studies[192]. Binding of AMP to AMPKγ is thought to promote phosphorylation of the crucial activation loop threonine (T172) in AMPKα, which is required for AMPK activity, largely through the suppression of phosphatase activity targeted at T172 (REF. 193). Mutation of some of these AMP-binding pockets in *AMPKγ2* lead to hypertrophic cardiomyopathy, which is associated with Wolff–Parkinson–White syndrome[194]. CTD, carboxy-terminal domain; GBD, GTPase protein-binding domain; NTD, amino-terminal domain.

LKB1 and metabolism of glucose and lipids

Although it is crucial in the suppression of diabetes, the reprogramming of glucose and lipid metabolism by LKB1-dependent kinases is also likely to be important for the growth and tumour-suppressive effects of LKB1. AMPK acutely inhibits fatty acid and cholesterol synthesis by phosphorylating the metabolic enzymes acetyl-CoA carboxylase 1 (ACC1) and HMG-CoA reductase (HMGCR)[51]. Activation of AMPK therefore provides an endogenous mechanism to inhibit HMGCR activity, which is akin to the pharmaceutical inhibition of HMGCR by the statin family of compounds[52]. As ACC1 and HMGCR are ubiquitously expressed, *LKB1*-deficient cells of all tissue types would be expected to show increased rates of lipid and cholesterol synthesis. Consistent with recent RNA interference (RNAi) studies which showed that ACC1 and fatty acid synthase (FASN) are essential for survival in several cultured tumour cell lines[53–55], chemical inhibitors of FASN and ACC1 have been shown to suppress the growth of prostate and lung cancer xenografts[56,57]. Indeed, a range of FASN inhibitors are being considered for clinical trails in cancer treatment[58], and it remains plausible that suppression of lipogenesis is an important part of the tumour suppressor function of LKB1. AMPK has also been suggested to

Steatosis
Excess intracellular lipid accumulation, which can occur, for example, in the liver of patients who are diabetic or obese.

acutely modulate glycolysis though the phosphorylation of multiple isoforms of phosphofructo-2-kinase (PFK2)[59,60]. The data are particularly compelling for the inducible PFK2 (PFKFB3) isoform, the expression of which is dramatically upregulated in some types of human cancer[61]. Indeed, genetic ablation of *Pfkfb3* in mouse lung fibroblasts suppresses KRAS-dependent transformation[62] and small-molecule inhibitors of PFKFB3 block the growth of lung cancer xenografts[63].



b

MARK, SAD or SIK substrates			AMPK substrates		
tau	S262	KSKIGSTENL	FOXO3a	S413	MQRSSSFPYT
MAP4	S1000	VSKKVSYSHT	HNF4a	S304	TKRLRSQVQV
PAR3	S889	LKKSSSLESL	AREBP	S470	AHRSKSHPAL
DVL2	S252	LERTSSFSSV	TBC1D1	S231	MRKSFSQPGL
KSR1	S404	LRRTESVPSDL	ACC1 and ACC2	S80 and S222	TRSSMSGLHL
CRTC2	S171	LNRTSSDSAL	HMGCR	S862	MIHNRSKINL
HDAC5	S259	LRKTASEPNL	PFKFB3	S461	LMRRNSVTPL
p300	S89	LLRSGSSPNL	raptor	S792	MRRASSYSSL
IRS1	S789	LRLSSSSGRL	TSC2	S1385	LSKSSSSPEL

Figure 2 | **Liver kinase B1-dependent signalling. a** | Liver kinase B1 (LKB1) complexed with its two regulatory subunits, STE20-related adaptor (STRAD) and MO25, directly phosphorylates and activates a family of 14 AMP-activated protein kinase (AMPK)-related kinases. In turn, these kinases phosphorylate several downstream substrates to mediate effects on cell polarity, metabolism and growth control. Well-established substrates of AMPK and its related family members are shown, and those for which further *in vivo* data are needed are shown with a question mark. It is important to note that many of the known substrates are expressed in a tissue-specific manner and might not explain the ubiquitous effects of LKB1 and its downstream kinases in all cell types. **b** | The sequences flanking the best-characterized phosphorylation site in each substrate are shown; residues that have been selected from *in vitro* peptide library and alanine-scanning peptide mutagenesis studies are highlighted. Importantly, to date there are no mutational data from human tumours to specifically support any of the downstream kinases, including the two AMPK catalytic genes, as being a particularly crucial target of LKB1 in tumour suppression. One confounding issue with the lack of mutations found in these downstream kinases is that there is a high level of redundancy among them, suggesting that loss of any one of these kinases might be compensated for by other family members, unlike LKB1, for which no other specific kinase has been shown to compensate for *in vivo*. ACC, acetyl-CoA carboxylase; AREBP, AICAR-responsive element-binding protein; CRTC2, CREB-regulated transcription coactivator 2; Dvl, dishevelled; FOXO3; forkhead box O3; HDAC5, histone deacetylase 5; HMGCR, HMG-CoA reductase; HNFα, hepatocyte nuclear factor-α; IRS1, insulin receptor substrate 1; KSR1, kinase suppressor of Ras1; MAP, microtubule-associated protein; MARK, MAP and microtubule affinity-regulating kinase; MYPT1, myosin phosphatase-targeting subunit 1; PAR3, partitioning defective 3; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PGC1α, PPAR-γ coactivator 1α; raptor, regulatory-associated protein of mTOR; SAD, snRNP assembly-defective; SIK, salt-inducible kinase; SNRK, SNF-related kinase; TSC2, tuberous sclerosis complex 2.

More broadly, LKB1-dependent kinases might also control cell growth and metabolism through the phosphorylation of widely expressed transcriptional co-activators. The p300 histone acetyltransferase (HAT)[64], several class IIa histone deacetyltransferases (HDACs)[65–67], and the CRTC (CREB-regulated transcription coactivator; previously known as TORC) family[68–71] have all been shown to be substrates of AMPK and related LKB1-dependent kinases (FIG. 2). Current data suggest that in response to distinct stimuli, subsets of LKB1-dependent kinases might target the same phosphorylation sites in these downstream effectors[72]. AMPK and its related kinases have been reported to phosphorylate class II HDACs and CRTCs, leading to their cytoplasmic sequestration and inactivation through 14-3-3 binding, in a similar manner to several other substrates of AMPK and its relatives. Although the best-studied transcriptional targets of class II HDACs and CRTCs are metabolic genes in muscle and liver, respectively, these proteins might have roles in a wider range of processes, such as cell proliferation and tumorigenesis[73,74]. AMPK has recently been shown to increase sirtuin 1 (SIRT1) activity by increasing cellular NAD^+ levels[75], resulting in the regulation of many downstream SIRT1 targets, including FOXO3 and peroxisome proliferator-activated receptor-γ co-activator 1 (PGC1; also known as PPARGC1A), both of which have also been proposed to be direct substrates of AMPK[46,76]. As SIRT1 is also implicated in tumorigenesis[77], this connection between AMPK and SIRT1 might further explain how nutrients control cell growth.

AMPK also suppresses mTOR-dependent transcriptional regulators to inhibit cell growth and tumorigenesis. Two mTORC1-regulated transcription factors involved in cell growth are the sterol-regulatory element-binding protein 1 (SREBP1) and hypoxia-inducible factor 1a (HIF1α). SREBP1 is a sterol-sensing transcription factor that drives lipogenesis in many mammalian cell types. mTORC1 signalling is required for nuclear accumulation of SREBP1 and the induction of SREBP1 target genes[78], and this can be inhibited by rapamycin or AMPK agonists[78,79]. Consistent with this, mice with a liver-specific *Lkb1* deletion had increased expression of SREBP1-target genes, hepatic lipid accumulation and steatosis[71]. Moreover, SREBP1 seems to be crucial for cell growth in both *D. melanogaster* and mammalian cells[78], suggesting that it might be an important target of LKB1, AMPK and mTOR signalling. Additional studies are needed to examine whether SREBP1 is upregulated in LKB1-deficient tumours and how important SREBP1 is for tumour formation in these conditions. HIF is a heterodimer composed of constitutive β-subunits (aryl hydrocarbon receptor nuclear translocator; ARNT) and α-subunits. The HIFα subunits are stabilized through the hypoxic inactivation of the von Hippel–Lindau (VHL) E3 ligase that targets them for destruction[80]. In addition to being increased through hypoxia, HIF1α protein levels are highly dependent on mTORC1 signalling. mTORC1 hyperactivation from mutations in oncogenes and tumour suppressors are sufficient to

Table 1| **Genetically engineered mouse models of liver kinase B1 function**

Mouse model	Targeted tissues	Phenotype	Significance	Refs
Lkb1$^{+/-}$	All tissues are heterozygous for *Lkb1*	Benign gastrointestinal hamartomas; multifocal osteoblastomas; paralysis	Genetic and histological phenocopy of Peutz–Jeghers syndrome; evidence for an unexpected function in bone?	115–118, 195
Lkb1$^{+/-}$;*Trp53*$^{-/-}$ and *Lkb1*$^{+/-}$;*Trp53*$^{+/-}$	All tissues heterozygous for *Lkb1* and null for *Trp53*	Gastrointestinal hamartoma development accelerated in *Lkb1*$^{+/-}$;*Trp53*$^{-/-}$ mice; hepatocellular carcinoma evident in one strain of *Lkb1*$^{+/-}$;*Trp53*$^{-/-}$ mice.	Loss of p53 cooperates with *Lkb1* heterozygosity. The reasons are unclear why hepatocellular carcinoma developed in only one experiment but not the other	196, 197
Lkb1 hypomorph	LKB1 only 10% active in all tissues	No tumours	It is unlikely that *Lkb1* is a haploinsufficient tumour suppressor for polyps, unless there is some form of compensation in this model	137
Lkb1 hypomorph;*Pten*$^{+/-}$	LKB1 only 10% active in all tissues; *Pten* heterozygous in all tissues	Increased onset of lymphomagenesis compared with *Pten* heterozygous mice	Low level activity of LKB1 is not sufficient to prevent lymphomagenesis	137
Sm22a–Cre;*Lkb1*$^{+/lox}$ and *Sm22a–Cre*;*Lkb1*$^{lox/lox}$	*Lkb1* expression knocked out in gastrointestinal smooth muscle cells	Benign gastrointestinal hamartomas	Polyps might arise as a result of mutation in smooth muscle cells and not epithelial cells	119
Cyp2a1–Cre;*Lkb1*$^{lox/lox}$	*Lkb1* expression knocked out in adult gastrointestinal epithelial cells	Altered differentiation of Paneth and goblet cells	LKB1 might have effects on epithelial cell differentiation in the gut. Is *Lkb1* deletion in the relevant cell population for polyp formation?	198
Lox–stop–lox–Kras^{G12D};*Lkb1*$^{lox/lox}$	Deletion in the lung epithelium only after inhalation of adeno–*Cre*	Mice develop aggressive non-small cell lung carcinomas that have either adeno, squamous or large cell features; widespread metastases	LKB1 loss synergises with KRAS activation in the lung; squamous lung cancer is not normally induced by *Kras* mutation, nor are widespread metastases	7
Lkb1$^{+/-}$ and *Lkb1*$^{lox/lox}$	Loss of *Lkb1* in endometrial epithelium induced by interuterine injection of adeno–*Cre*	Invasive endometrial adenocarcinoma	Endometrium might be highly sensitive to loss of LKB1	121
Cyp1a1–Cre;*Lkb1*$^{lox/lox}$	Prostate epithelium	Hyperpasia and neoplasia of the prostate epithelium	Sex hormone-regulated growth might be affected	199
Lkb1$^{+/-}$ and *K14–Cre*;*Lkb1*$^{lox/lox}$	Skin epithelium; DMBA administered to the skin	Squamous cell carcinoma of the skin (and occasionally the lung)	*Lkb1* loss might be synergistic with DMBA-induced mutation of *Hras*	124
Lkb1$^{+/-}$ and *Pdx1–Cre*;*Lkb1*$^{lox/lox}$	Pancreatic precursors	Benign pancreatic cystoadenomas	Altered polarity and developmental defects	200

Cyp1a1, cytochrome P450 1A1; DMBA; 7,12-dimethylbenz(a)anthracene; *K14*, keratin 14; *Lkb1*, liver kinase B1; *Pdx1*, pancreatic and duodenal homeobox 1; *Sm22a*, smooth muscle 22α (also known as transgelin).

promote HIF1α protein levels and the expression of its downstream targets in mouse cancer models and cells *in vitro*[81]. Well-established HIF1 transcriptional targets that contain hypoxia-responsive elements in their promoters include angiogenic factors, such as vascular endothelial growth factor A (VEGFA) and angiopoetin 2 (ANG2), several glycolytic enzymes and multiple members of the glucose transporter (GLUT) family[82]. In this manner, HIF1α activation in tumours might be responsible for the Warburg effect — the propensity of tumour cells to rely on glycolysis instead of oxidative phosphorylation[83]. Indeed, this regulation of glucose metabolism by HIF1α contributes to tumorigenesis in multiple settings[84,85]. Consistent with earlier studies in TSC-deficient fibroblasts[86], we have recently shown that the levels of HIF1α and its targets GLUT1 and hexokinase are increased in LKB1-deficient and AMPK-deficient fibroblasts in a rapamycin-reversible manner[36]. Similarly, the epithelia of gastrointestinal hamartomas from patients with PJS or *Lkb1*$^{+/-}$ mice (TABLE 1) also show increased expression of HIF1α- and HIF1-target genes compared with the surrounding

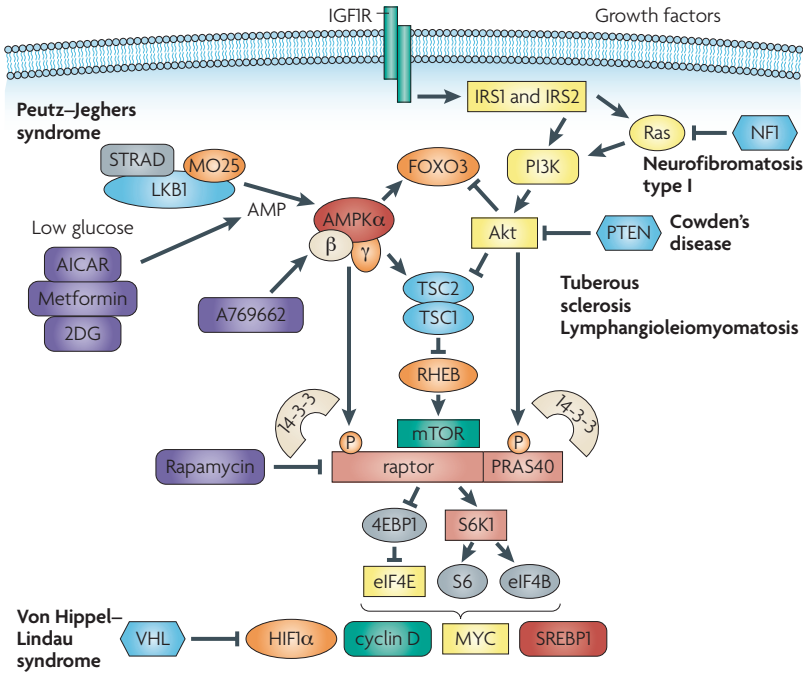


Figure 3 | **AMP-activated protein kinase and PI3K signalling converge to antagonistically regulate several downstream effectors, including mTOR complex 1.** Many inherited hamartoma and cancer predisposition syndromes, such as Peutz–Jeghers syndrome and Cowden's disease, commonly show hyperactivation of mTOR complex 1 (mTORC1) or hypoxia-inducible factor 1α (HIF1α). AMPK suppresses mTOR-dependent transcriptional regulators (such as cyclin D1 and MYC) to inhibit cell growth and tumorigenesis. Two mTORC1-regulated transcription factors involved in cell growth are the sterol-regulatory element-binding protein 1 (SREBP1) and HIF1α. The HIFα subunits are stabilized through the hypoxic inactivation of the von Hippel–Lindau (VHL) E3 ligase that targets them for destruction[80]. However, HIF1α protein levels are highly dependent on mTORC1 signalling, and mTORC1 hyperactivation from mutations in oncogenes (shown in yellow) and tumour suppressors (shown in blue) are sufficient to increase HIF1α protein levels. Conditions that lower intracellular ATP levels; for example, low glycolytic rates from low glucose concentrations or inhibitors, such as 2-deoxyglucose (2DG), or oxidative phosphorylation inhibitors, such as metformin and related biguanides (shown in purple), will lead to activation of AMPK in a liver kinase B1 (LKB1)-dependent manner. Aminoimidazole carboxamide ribonucleotide (AICAR) is a precursor of zinc metalloproteinase (ZMP), which acts as an AMP mimetic and is thought to directly bind the AMP-binding pockets of the AMPKγ subunit. A769662 is the only known small molecule that directly binds AMPK, inducing its activity, although it is not currently known at which region the compound binds on the AMPK heterotrimer. 4EBP1, translation initiation factor 4E-binding protein 1; eIF4, eukaryotic translation initiation factor 4; FOXO3; forkhead box O3; IGF1R, insulin-like growth factor 1 receptor; IRS1, insulin receptor substrate; NF1, neurofibromin; raptor, regulatory-associated protein of mTOR; RHEB, Ras homologue enriched in brain; S6K1, ribosomal protein S6 kinase β1; STRAD, STE20-related adaptor; TSC, tuberous sclerosis complex.

normal tissue, suggesting that HIF1α might be a relevant target downstream of LKB1 deficiency in PJS[36]. The increase in glucose uptake in tumours from patients with PJS could also be used to guide surgical resection of hamartomas in the gastrointestinal tract. FDG-PET ([18F] 2-fluoro-2-deoxy-D-glucose positron emission tomography) imaging studies on *Lkb1*$^{+/-}$ mice showed that their gastrointestinal hamartomas are specifically labelled in a rapamycin-sensitive manner. Given this result, it will be interesting to examine whether the presence of *LKB1* mutations dictates the level of FDG-PET signal in other tumour models, particularly in NSCLC and cervical cancer.

LKB1–AMPK and cell polarity

In *D. melanogaster*, disruption of *lkb1*, *par1* or *snfla* (which encode AMPK) results in polarity defects during embryogenesis[87–90] and oogenesis[91]. In mammalian cells, inducible activation of LKB1 is sufficient to promote full polarization of tumour cells, including apical and basolateral cell sorting, an actin cap and a full brush border, even in the absence of cell–cell contacts[92]. In cultured hippocampal neurons, overexpression of LKB1 induces multiple axons, and depletion of LKB1 or its subunit STRAD using RNAi blocks axonal differentiation[93]. Consistent with these findings, tissue-specific deletions of *Lkb1* or brain-specific kinase 1 (*Brsk1*) or *Brsk2* (downstream targets of LKB1 and orthologues of *C. elegans* *sad-1*, which encodes a kinase) in mice result in loss of axonal specification during neuronal polarization in the developing mammalian cerebral cortex[94]. It is important to note that LKB1 does not seem to be required for polarization of all tissues, as several tissue-specific deletions of *Lkb1* in mice do not show disruptions of cellular polarity or tissue organization[95]. The requirement of LKB1 for the establishment of polarity instead of the maintenance of polarity is an additional consideration for the interpretation of these experiments. Cell polarity is known to be established through the action of several conserved antagonistic polarity protein complexes, and LKB1 and its downstream MARKs contribute to this regulation (BOX 1). LKB1 might also influence cell polarity and migration through several substrates of its downstream kinases that are involved in cytoskeletal remodelling. For example, MARK-dependent phosphorylation of MAPs is thought to play a part in cell migration[96] and might be relevant to the increased metastatic nature of NSCLC lung tumours that specifically lack LKB1 (REF. 7). MARKs phosphorylate serine residues in the microtubule-binding domain of MAPs, resulting in increased dynamic instability of cellular microtubules[97].

Another set of conserved MARK substrates are the dishevelled (Dvl) proteins, which are key mediators of the Wnt signalling pathway[98]. Although MARK phosphorylation of Dvl regulates the membrane localization of Dvl, this is not required for canonical Wnt signalling in *Xenopus laevis*[99], and the MARK phosphorylation sites in Dvl do not seem to be required for the MARKs to affect Wnt signalling[99,100]. This suggests that there must be additional unidentified MARK substrates involved in Wnt signalling. Interestingly, canonical and non-canonical Wnts were recently shown to induce cytoskeletal remodelling through Dvl binding to the Par complex, promoting atypical PKC-mediated inactivation of the MARKs[101–103]. Wnt-dependent signals, which promote tumorigenesis in several tissues, including colon and breast cancer, might therefore modulate LKB1-dependent signalling through multiple mechanisms, and LKB1-dependent signals might also modulate Wnt signalling (FIG. 4). AMPK has also recently been reported to modulate cell polarity in *D. melanogaster* and mammalian cells. AMPK activation in Madin–Darby canine kidney (MDCK) cells led to an increase in tight junctions[104,105], and treatment of a colon cancer cell line with the glycolytic inhibitor 2-deoxyglucose led to an AMPK-dependent increase in the

number of polarized cells[89]. In addition, LKB1 and its regulatory subunit STRAD localize to adherens junctions in MDCK cells in an E cadherin-dependent manner[106]. Loss of E cadherin leads to specific loss of AMPK activation at adherens junctions. Studies of AMPK mutants in *D. melanogaster* showed mislocalization of the Par complex as well as other polarity markers, including loss of myosin light chain (MLC) phosphorylation[89]. It was suggested that MLC might be a downstream substrate of AMPK; this seems unlikely as the phosphorylation sites in MLC do not conform to the optimal AMPK substrate motif found in all other established *in vivo* AMPK substrates. However, AMPK and its related family members have been reported to modulate the activity of kinases and phophatases that regulate MLC — MLC kinase (MLCK)[107] and myosin phosphatase-targeting subunit 1 (MYPT1)[108] — so the full molecular details of the mechanism require further study. Given the overlapping substrate specificity of AMPK and its related kinases (FIG. 2), it seems likely that AMPK might control cell polarity by targeting some of the same substrates as other AMPK family members, such as the MARKs, which are phosphorylated in other conditions.Finally, it was recently shown that LKB1 promotes brush border formation on the apical surface of epithelial cells by the activation of the MST4 kinase. MST4 binds the LKB1 partner MO25 (also known as CAB39), and this interaction is conserved in budding yeast[109]. LKB1-dependent polarization resulted in MST4 translocation and subsequent phosphorylation of the cytoskeletal linker protein ezrin (EZR). This function of MST4 was needed for brush border induction but not other aspects of polarization.Whether the control of cell polarity plays any part in LKB1-dependent tumour suppression also awaits further study. A recent study suggested that this process might be important by showing that knockdown of *LKB1* using RNAi in MCF10A mammary acini in three-dimensional culture led to a loss of polarity and promoted oncogenic *MYC*-dependent cell proliferation[110], an effect that cannot be seen in standard tissue culture plates[111–113]. Dissection of the role of LKB1 in cell polarity is therefore perhaps best examined in the context of mouse models of *Lkb1* deficiency.

Box 1 | **Polarity protein complexes**

Studies across a wide range of metazoans have shown that the molecular control of cell polarity is commonly established through the opposing function of a small number of polarity protein complexes that mutually exclude the localization of each other[172]. In addition to serine–threonine liver kinase B1 (LKB1) and the microtubule-activating protein and microtubule affinity-regulating kinases (MARKs; also known as par-1 kinases), other highly conserved polarity genes include *PAR3* and *PAR6*, the products of which form a quaternary complex with the small GTPase cell division control 42 (CDC42) and atypical PKC (aPKC) subfamily of kinases (referred to as the Par complex). The binding of CDC42 to the Par complex results in activation of aPKC, which directly phosphorylates the MARK family on a conserved carboxy-terminal threonine, leading to their association with 14-3-3 proteins and exclusion from the apical domain of the cell[178–180] (FIG. 4). Reinforcing the mutual exclusion of the polarity complexes, the MARKs have been reported to directly phosphorylate and cause relocalization of the discs large (DLG) polarity proteins[181] and the PAR3 scaffolding protein[182]. Whether this proposed mutual exclusion of the MARKs and Par complex can explain the observed effects of LKB1 loss on glycogen synthase kinase 3 and CDC42 activity in different settings[183,184], including non-small cell lung cancer cell lines[185], remains to be determined.

LKB1 and mouse models of cancer

Consistent with its regulation of cell growth, metabolism and polarity, genetic studies on the loss of function of LKB1 in mice have identified many cancerous phenotypes (TABLE 1). As in patients with PJS, mice heterozygous for *Lkb1* develop gastrointestinal polyposis[114–118]. Strikingly, mice in which *Lkb1* is specifically deleted in gastrointestinal smooth muscle cells also develop polyps that are similar to those in $Lkb1^{+/-}$ mice[119]. These mice had alterations in transforming growth factor-β (TGFβ) signalling, implicating this pathway in hamartoma formation[120], and have raised the possibility that loss of LKB1 in the smooth muscle compartment and not the epithelial cells might be the initiating event in tumorigenesis. Future studies are needed to further test this model. In addition to gastrointestinal hamartomas, patients with PJS have a predisposition to many other malignancies, including breast, ovarian, endometrial and pancreatic tumours, and some of these tumours have been studied in specific *Lkb1* mouse models (TABLE 1). Given the recent discovery of prevalent *LKB1* somatic mutations in cervical cancer and their association with poor prognosis[8], it is particularly notable that the deletion of *Lkb1* in the endometrial epithelium of female mice results in highly invasive adenocarcinomas[121].

As *LKB1* is frequently co-mutated with *KRAS* in NSCLC[122,123], mice bearing a conditional activated allele of *Kras* were crossed with mice bearing a conditionally inactivated allele of *Lkb1*. There was a dramatic increase in tumour incidence and metastasis in the $Kras;Lkb1^{lox/lox}$ mice, resulting in a rapid acceleration of death (25 weeks for *Kras* alone compared with 10 weeks for $Kras;Lkb1^{lox/lox}$)[7]. Furthermore, these mice develop all subtypes of NSCLC, as seen in humans, including squamous lung tumours, which have not been previously observed in any genetic mouse model of lung cancer. Mechanistically, whether loss of LKB1 allows a distinct cell population to grow out and form squamous tumours, or whether LKB1 loss affects a lung stem cell compartment and alters lung stem cell differentiation has yet to be investigated. Loss of LKB1 in skin keratinocytes was also recently reported to promote the development of squamous cell carcinomas, which was greatly accelerated by DMBA (7,12-dimethylbenz(a)anthracene) treatment[124]. 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 tumour cells.

Therapeutic implications

AMPK agonists as cancer therapeutics. Because of its long-established roles in various aspects of metabolic physiology, AMPK has received a great deal of pharmaceutical interest as a target for type 2 diabetes and other aspects of the metabolic syndrome[125]. Metformin (Glucophage) is the most widely used type 2 diabetes drug and is thought to act by decreasing hepatic gluconeogenesis[126]. 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[127]. Indeed, this pathway is required for the therapeutic ability of metformin to lower

blood glucose levels[71]. More recently, as metformin has been more widely prescribed for different diseases (for example, the treatment of insulin resistance in individuals with polycystic ovary syndrome) polymorphisms in *LKB1* have been found in metformin non-responders[128]. More investigation is needed to determine the effect of these polymorphisms. Similarly, genetic polymorphisms in the cell surface transporter organic cation transporter 1 (*OCT1*), which is required for efficient metformin uptake in hepatocytes, have been shown to underlie metformin resistance in some patients with type 2 diabetes[129].

As AMPK activation not only reprogrammes metabolism, but also enforces a metabolic checkpoint on the cell cycle through its effects on p53 and mTORC1 signalling, this suggests that AMPK-activating drugs might be useful as cancer therapeutics. Interestingly, well before the mode of action or key targets of metformin were known, it had been shown to suppress naturally arising tumours in transgenic mice and in carcinogen-treated rodent cancer models[130,131]. More recently, metformin has been shown to inhibit the growth of a wide range of tumour cells in culture in an AMPK-dependent manner[132,133], and AMPK activation by metformin or aminoimidazole carboxamide ribonucleotide (AICAR) suppresses the growth of tumour xenografts[134–136]. Similarly, treatment of embryonic stem cells with metformin results in growth suppression, an effect that is lost in LKB1-deficient embryonic stem cells[137]. Given the known pharmacokinetics and widespread long-term clinical use of metformin, its potential use for chemotherapy deserves further attention. Phenformin is a more potent inhibitor of mitochondrial complex I and, consequently, more potently activates AMPK than metformin[138]. Despite the withdrawal of phenformin from clinical use owing to the likely on-target side effect of fatal lactic acidosis[139], it might be useful as an anticancer agent given that the dosing and duration of its use for cancer would be distinct from those used for diabetes. The anti-tumour efficacy of metformin has been directly compared with that of either phenformin or the AMPK-binding[140] small molecule A769662 (REF. 141) in *Pten*$^{+/-}$ mice that spontaneously develop lymphomas. Although all three compounds resulted in delayed tumour onset, phenformin and A769662 showed greater efficacy, which correlated with their ability to activate AMPK and suppress mTORC1 in a larger number of tissues *in vivo* than metformin[137]. Perhaps an additional key to the success observed in this study is the fact that tumours initiated through loss of *Pten* have PI3K activation, making mTORC1 hyperactivation one of the biochemical initiating events for this tumour type and increasing the effect of suppression of mTORC1 from endogenous AMPK activation. These data also suggest a possible therapeutic window for the use of AMPK agonists to treat tumours that arise in patients with TSC or for tumours that show hyperactivation of mTORC1 by other genetic lesions. The fact that a direct AMPK-binding compound also gave promising results further suggests that AMPK is a key target of the biguanides in tumour reduction.

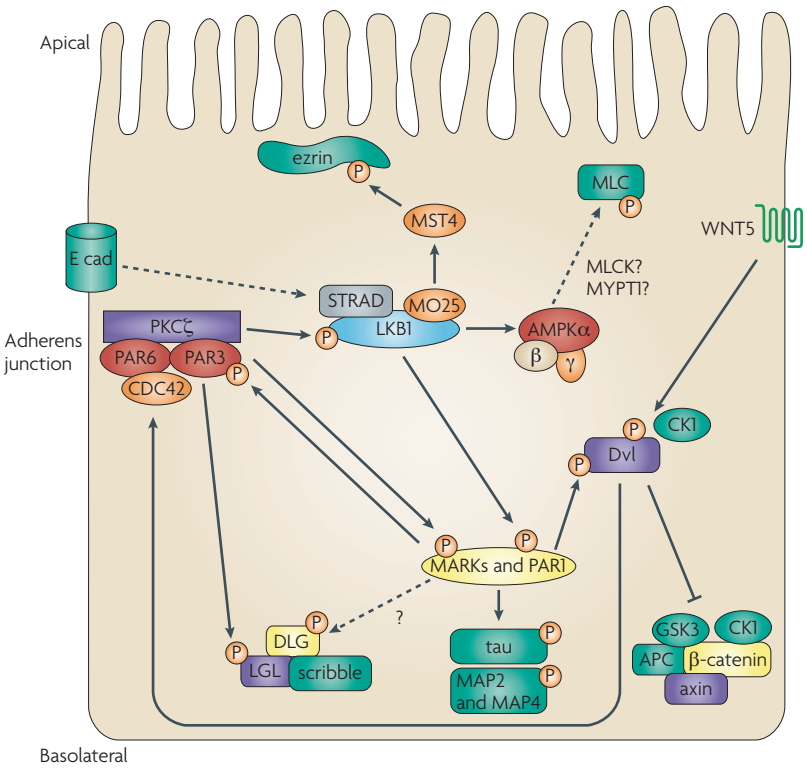


Figure 4 | **Control of cell polarity by liver kinase B1-dependent signalling.** The partitioning defective (Par) complex, which is composed of an atypical PKC family member, the PAR3 scaffold, the cell division control 42 (CDC42)-binding PAR6 and CDC42, phosphorylates many downstream polarity proteins, including liver kinase B1 (LKB1), the microtubule-associated protein (MAP) and microtubule affinity-regulating kinase (MARK) family, and lethal giant larvae (LGL). LKB1 also requires a signal from E cadherin (E cad) to be recruited and competent to phosphorylate AMP-activated kinase (AMPK) at the adherens junction. LKB1-dependent AMPK activation is known to modulate the phosphorylation state of myosin light chain (MLC) in *Drosophila melanogaster* mutants; this might be mediated through indirect regulation of the kinase (MLC kinase; MLCK) and phosphatase (myosin phosphatase-targeting subunit 1; MYPT1) for MLC. In turn, LKB1-dependent MARKs phosphorylate the PAR3 scaffold, thereby leading to the mutual exclusion of the Par complex and the MARKs in the cell. It is well established that MARKs can also phosphorylate MAPs, including tau, MAP2 and MAP4, and have been reported to phosphorylate disks large homologue (Dlg) and dishevelled (Dvl) proteins in some contexts. APC, adenomatous polyposis coli; CK1, casein kinase 1; GSK3, glycogen synthase kinase 3; STRAD, STE20-related adaptor.

Given the number of patients with type 2 diabetes worldwide who are taking metformin daily (>100 million), epidemiologists have begun examining the effect of metformin on cancer incidence. Initial studies showed that patients with diabetes taking metformin show a statistical reduction in tumour burden compared with patients taking any alternative drug[142,143]. Similarly, a recent study of breast cancer in patients with type 2 diabetes who were taking metformin reported a significant increase in pathological complete response rates (that is, no evidence of cancer cells in breast tissue or draining lymph nodes)[144], and a large Phase III clinical trial of metformin as an adjuvant in breast cancer for patients with or without diabetes is in development[145]. Importantly, compounds that activate AMPK will not only affect tumour incidence through cell-autonomous effects on cell growth downstream of AMPK, but perhaps also through non-cell autonomous effects of lowering plasma insulin levels, which itself contributes to cancer risk and incidence[146]. Many additional epidemiological studies are required to determine whether there is a clear tumour suppressive effect of prolonged use

of metformin and, if so, whether tumours that arise in specific tissues or that have specific oncogenic lesions will show the greatest potential response. Interestingly, the OCT1 transporter, which is crucial for effective metformin transport into hepatocytes, shows a limited tissue distribution[129] that is consistent with the pattern of AMPK activation in mice treated with metformin[137]. By contrast, phenformin shows potent activation of AMPK[137] in a wider range of tissues, indicating that the effect of metformin might be restricted compared with phenformin. However, a recent study showed that metformin was effective in treating a mouse model of endometrial hyperplasia and could reduce mTORC1 signalling[147]. Whether this effect was due to direct activation of AMPK in the endometrium or to reduced levels of circulating insulin and insulin signalling was not examined. Going forward, further attention needs to be paid to whether the effects of metformin in mice and in human epidemiology studies are as a result of reduced insulin levels owing to AMPK activation in liver or as a result of AMPK activation in tumour cells, which leads to suppression of their growth. These effects need not be mutually exclusive and both are likely to contribute.

Even with effective targeting and activation of AMPK in tumour cells, as with other targeted therapeutics, AMPK-activating drugs are likely to be most useful against tumours of specific genotypes or in combination with other targeted therapeutics. Tumour cells lacking LKB1 are hypersensitive to apoptosis in culture following treatment with energy stress-inducing agents; presumably this phenotype originates from an inability to restore ATP levels owing to AMPK deficiency[4,37,148,149]. Similarly, fibroblasts lacking TSC2 or p53 are also sensitive to apoptosis induced by energy stress[28–30,40], and metformin and AICAR both preferentially killed isogenic colon cancer xenografts that lacked p53 compared with xenografts that had intact p53 function[135]. Although energy stress can promote apoptosis in cells with a defective AMPK pathway, cells with an intact AMPK pathway can survive this stimulus[47,150,151]. Treatment of tumours that have intact AMPK function with agents that induce energy stress could therefore lead to the prolonged survival of tumour cells. This is consistent with the ability of AMPK to promote the survival of cells that are faced with metabolic stress that is imposed by oncogenic activation[115,152]. Therefore, the transient inactivation of AMPK might chemosensitize some tumours in a similar way to drugs that target the DNA damage checkpoint[153].

Therefore, defining which oncogenic genotypes (such as loss of p53 or LKB1) sensitize tumours to AMPK-activating drug treatments in more refined genetically engineered mouse tumour models for individual tumour types (such as lung and mammary tumours) is an important goal for future studies.

Rapamycin as a therapeutic for hamartomas and other LKB1-deficient tumours. Mutations in the *PTEN*, *NF1*, *TSC2* or *LKB1* tumour suppressor genes are responsible for several inherited cancer syndromes, which are collectively referred to as phakomatoses. These syndromes all have overlapping clinical features, which include the development of hamartomas and aberrant pigmentation defects. Given that each of these tumour suppressors function upstream of mTORC1 (FIG. 3), the underlying hypothesis is that inactivation of these tumour suppressors in individual cells leads to cell-autonomous hyperactivation of mTORC1, ultimately resulting in tumours that are reliant on mTORC1 signalling. Over the past 5 years, rapamycin analogues have been examined in spontaneously arising tumours in *Pten*$^{+/-}$ (REF. 154), *Nf1*$^{+/-}$ (REF. 155), *Tsc2*$^{+/-}$ (REF. 156), *Lkb1*$^{+/-}$ (REFS 36,157,158) and activated Akt[84] transgenic mice, and tumours in these mice have proven to be responsive to this approach.

These encouraging preclinical results have helped spur ongoing Phase II and III clinical trials for rapamycin analogues[159–162]. These data suggest that hamartoma syndromes involving hyperactivation of mTORC1 might be particularly responsive to rapamycin analogues as a single agent, although the effects might be cytostatic rather than cytotoxic[161]. Perhaps new, targeted inhibitors directed at the kinase domain of mTOR will produce greater therapeutic responses with targeted cytotoxicity or perhaps kinase inhibitors that inactivate both mTOR and PI3K would be even more effective than rapamycin analogues, as PI3K provides a survival signal in most epithelial cell types.The number of patients with inherited hamartoma syndromes is dwarfed by the number of people with sporadic lung tumours that contain *LKB1* mutations. However, the predicted effectiveness of mTORC1 inhibitors against these tumours is unclear given that most of these tumours have mutated *KRAS* in addition to loss of LKB1, which might activate survival pathways other than mTORC1. Whether mTORC1 inhibitors might be useful in the treatment of *LKB1*-mutant tumours of different tissue origins remains to be determined.

Unanswered questions

The existence of a nutrient-regulated tumour suppressor pathway that couples cell growth to glucose and lipid metabolism raises a number of intriguing predictions and unanswered questions. For example, do environmental factors, such as diet and exercise that contribute to physiological AMPK activation, modulate tumorigenic risk through mTORC1 suppression? It is clear from many epidemiology studies that cancer risk is correlated with metabolic syndrome, obesity or type 2 diabetes[163]. This association might be due to increased cell proliferation through the hyperactivation of mTORC1 downstream of altered LKB1–AMPK signalling. The identity of the cell types that are most sensitive to the growth suppression effects of AMPK and LKB1 might identify those lineages in which cell growth is most tightly coupled to dietary conditions. Conversely, exercise and caloric restriction, each of which activates AMPK in some cell lineages, can lower overall cancer risk and improve cancer prognosis[164]. The mammalian cell types in which exercise and caloric restriction suppress cell growth and reduce cancer risk have yet to be identified. A recent study showed that AMPK was activated and mTORC1 signalling was suppressed in some rodent tissues in a dose-dependent manner by increasing amounts of dietary restriction[165]. Conversely, a high

fat diet was observed to increase mTOR and decrease AMPK activity in some mouse tissues[166]. Finally, lower expression levels of metabolic hormones, including the adipokine adiponectin — which is a key activator of AMPK in some tissues — have been shown to correlate with increased risk for breast, endometrial, prostate and colon cancer[167,168]. Strikingly, the incidence of colonic polyps in a colorectal cancer mouse model that lacked adiponectin or the adiponectin receptor 1 (ADR1), was significantly increased, and this correlated with loss of AMPK signalling and increased mTORC1 in the colonic epithelium[169]. These effects were only observed in animals on a high fat diet, further reinforcing the concept that the metabolic status of the cells and the organism will dictate the conditions in which LKB1 is most effective in tumour suppression.Whether the endogenous metabolic checkpoint imposed by AMPK must be suppressed to allow tumorigenic progression is also unclear. Melanoma cell lines that express oncogenic BRAF do not activate AMPK following energy stress owing to hyperphosphorylation of LKB1 at ERK- and RSK-phosphorylation sites[170]. Moreover, the levels of *AMPKα2* mRNA in breast and ovarian cancers are suppressed by oncogenic PI3K signals[171], suggesting another route through which AMPK signalling can be inhibited. Therefore, there is evidence that oncogenic pathways can downregulate LKB1 and AMPK through a range of mechanisms. It is also unclear when selection against the LKB1–AMPK pathway occurs, but it is conceivable that limitations on glucose and oxygen diffusion in pre-angiogenic tumours will result in growth inhibition, possibly owing to the activation of an AMPK-mediated metabolic growth checkpoint. Whether endogenous AMPK signalling is truly part of the pre-angiogenic checkpoint is a crucial question. Furthermore, whether pre-angiogenic tumours that lack LKB1 or AMPK continue to proliferate faster than their AMPK-containing counterparts but then succumb to apoptosis or necrosis owing to the inevitable energy shortage remains to be seen. The role and requirement for AMPK in these processes and overall tumour suppression is perhaps best addressed genetically through the deletion of AMPK subunits in the context of different well-studied mouse models of tumorigenesis.

Despite the evidence supporting a role for AMPK as a metabolic checkpoint in the cell, key mechanistic questions remain regarding which of the kinases downstream from LKB1 and which of their substrates are required for the tumour suppressor activity of LKB1 in different tissues. The regulation of mTORC1 and p53 by AMPK makes it a likely contributor to LKB1-dependent tumour suppression. However, control of cell polarity is also known to play a part in tumorigenesis[172,] and suppression of MARKs by the *Helicobacter pylori* cytotoxicity-associated immunodominant antigen (CagA) is thought to be essential for the pathogenic disruption of gastric epithelial polarity and tumour promotion caused by this protein[173]. Currently there are minimal mutational data from human tumours to specifically support any single LKB1-dependent kinase as the crucial target for LKB1 in tumorigenesis. There is a high level of redundancy among these kinases, suggesting that, in many tissues, the loss of any one kinase might be compensated for by other family members.

The potency of LKB1 as a tumour suppressor probably derives from its control of multiple growth suppressive pathways. For example, combined loss of LKB1 with KRAS in the mouse lung epithelium causes three discrete phenotypes: accelerated tumour progression and tumour growth; the appearance of a new tumour type, squamous carcinomas; and a dramatic increase in the number of metastases. Although AMPK and mTORC1 signalling might play a part in the growth component of this acceleration, it also seems probable that the loss of cell polarity and increased cytoskeletal signalling that occurs after loss of MARK activity affects the unique metastatic nature of the *LKB1*-deficient tumours. The appearance of new tumour types might also reflect de-differentiation through transcriptional reprogramming downstream of AMPK and several of its related family members. AMPK has also been shown to modulate other tumour suppressive mechanisms, including the promotion of autophagy[174] and cellular senescence[175] in low energy conditions. The absolute requirement for AMPK or LKB1 in the induction of senescence or autophagy in different physiological and pathological contexts in an intact organism remains to be fully investigated.

Another important question is whether LKB1 or AMPK deregulation often contributes to the Warburg effect. Studies from cell culture and targeted mouse knockouts have shown that mutations in the oncogenes and tumour suppressors that drive tumorigenesis stimulate HIF1α[176]. Indeed, HIF1α and its target genes are upregulated in *LKB1*- *AMPK*-, and *TSC*-deficient fibroblasts even in normoxic conditions, indicating that loss of any one of these genes is sufficient to confer activation of the full HIF1α transcriptional programme and therefore alter cell metabolism[36,177]. Immunohistochemistry on gastrointestinal tumours from patients with PJS and *Lkb1*$^{+/-}$ mice shows that both types of tumours contain increased levels of HIF1α and its target GLUT1, and that in *Lkb1*$^{+/-}$ mice, these tumours can be visualized using FDG-PET, despite their benign nature[36]. These observations further prompt an examination of physiological or pathological contexts in which LKB1 or AMPK normally suppress HIF1α, and whether their inactivation is commonly involved in the glycolytic switch that occurs in most tumours. Given the regulation of the LKB1–AMPK pathway by hormones, exercise and diet, future studies should address whether LKB1 or AMPK mediate changes in tumour metabolism and FDG-PET imaging following behavioural or hormonal intervention. Whether NSCLC and cervical cancers that express mutant LKB1 show altered FDG-PET, and whether this characteristic can be used to direct therapeutic interventions in different patient populations, will be important aims for future studies. Regardless, the development of new serum and tissue biomarkers that represent the activation states of LKB1 and AMPK will lead to better optimization of future clinical trials that aim to improve the efficacy of targeted therapeutics. Although these questions and

many others will take years to fully address, the discovery of this highly conserved pathway has already led to fundamental insights into the mechanisms through which all eukaryotic organisms couple their growth to nutrient conditions and metabolism. A deeper understanding of the key components of the AMPK pathway will not only lead to future therapeutic targets for cancer and diabetes, but will identify the minimal number of steps that are required to suppress cell growth and reprogramme metabolism.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Brsk1 | *Brsk2* | *CDKN1A* | *NF1* | *OCT1* | *Pten* | *SESN1* | *SESN2*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary
[18F] 2-fluoro-2-deoxy-D-glucose | metformin | rapamycin
UniProtKB: http://www.uniprot.org
4EBP1 | ACC1 | ANG2 | ATM | ATR | BRAF | CagA | CAMKK2 | cyclin D1 | DNA-PK | E cadherin | EZR | FASN | FOXO3a | GLUT1 | HAT | HIF1α | HMGCR | KRAS | LKB1 | MLCK | MO25 | MST4 | MYPT1 | p27 | p53 | PAR-1 | PFKFB3 | PGC1 | raptor | RHEB | rictor | S6K1 | SIRT1 | SREBP1 | STRAD | TSC1 | TSC2 | VEGFA

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Reuben J. Shaw's homepage:
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