Review

LKB1 loss of function studied in vivo

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Abstract

Recent developments have placed the serine/threonine kinase LKB1 on the crossroads linking energy metabolism, cell structure and cancer progression and that its deletion can affect tumorigenesis, metastasis, cell adhesion and polarity. LKB1 can regulate a host of different functions which all have potential to impact upon the initiation and progression of neoplastic disease. To understand the phenotypic consequences of LKB1 loss in a range of different settings, a number of animal models of loss of function have been generated and analyzed. In this review we summarize recent data generated from a range of these models, which reveal clear tissue specific differences in LKB1 function in vivo and in the consequences of its loss.

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1. Background

LKB1/STK11 is a serine/threonine kinase that controls diverse cellular processes through phosphorylation of a conserved threonine residue in a family of kinases [1,2]. As a consequence of the broad range of LKB1 substrates, LKB1 potentially impacts on a very wide range of cellular processes. Key amongst these functions is the activation of AMPK kinase when cellular energy levels are depleted to restore the ATP/AMP ratio through promotion of ATP production and blocking of its consumption [2]. However, LKB1 has been implicated in many other processes relevant to tumour initiation and progression. Hence, in concert with AMPK, LKB1 restricts cell growth by activation of the TSC1/TSC2 complex and subsequent blocking of Rheb and mTOR [3], a pathway which is also suppressed by AMPK via phosphorylation of the regulatory-associated protein of mTOR (raptor) [4]. AMPK itself not only affects metabolism and cell growth but also stabilizes contacts between cells by tight junction assembly [5]. LKB1 has also been implicated in controlling cell polarity, mainly through the targeting of four members of the MARK (microtubule affinity-regulating kinases) and SAD (BRSK) families [6,7]. Furthermore, LKB1 has been shown to influence cell adhesion, cell death, detachment and invasion, for example through regulation of NUAK1 kinase activity, which is important for cytoskeletal motor protein regulation [8], and by regulation of SIK1-dependent anoikis [9].

LKB1 normally functions inside a large protein complex that also includes STRADα and MO25 [10,11]. In this structure, MO25 stabilizes the complex and STRADα promotes the active conformation of LKB1, stimulating catalytic activity and facilitating LKB1 export from the nucleus to the cytoplasm [12,13]. Interestingly, LKB1 has a dual nuclear-cytoplasmic function and it is inactive when its localization restricted to the nucleus only [14].

Mammalian LKB1 is highly homologous to its counterparts in Drosophila, Xenopus (XEEK1) and Caenorhabditis elegans (PAR-4), suggesting a conserved role thorough evolution. The gene was initially identified in C. elegans [15] and Xenopus [16], but it attracted considerable interest when germline inactivating mutations of LKB1 were linked to Peutz–Jeghers syndrome (PJS), a syndrome characterized by benign gastrointestinal polyps and increased susceptibility to a large variety of malignancies [17]. The tumour suppressor role of LKB1 has since been underscored by the observation of LKB1 somatic mutations in sporadic lung cancer [18], and also by the more recent suggestion that it may act as a major cervical tumour suppressor [19].

2. LKB1 loss and human disease

2.1. Inherited Lkb1 loss and Peutz–Jeghers syndrome

The LKB1/STK11 gene was first cloned as a result of a comparative genomic hybridization analysis performed on patients suffering from Peutz–Jeghers syndrome (PJS) [17,20,21]. Initially, chromosome 19p13.3 locus mutations were reported to be the primary cause of PJS [20], and following an intense analysis of this region the LKB1 gene was pinpointed [17,21]. Subsequently, the majority of PJS cases have been confirmed as being characterized by germline LKB1 mutations [22,23].
PJS is a rare autosomal dominant inherited condition characterized by multiple polyps in the gastrointestinal tract that develop predominately in the small intestine, but also with fewer lesions arising in the large intestine and stomach [17,24–27]. The most distinguishing manifestation of PJS is mucocutaneous pigmentation, which is present in childhood but can disappear later. These melanin pigment spots can affect lips, the buccal mucosa as well as the face, forearms, anus, fingers, toes and intestinal mucosa [28]. PJS gastrointestinal polyps develop early in life and can lead to chronic bleeding, abdominal pain and recurrent bowel obstructions [29]. The polyps have distinct histopathological characteristics, usually being on stalk-like growths; with the intestinal crypts and gastric pits/glands often elongated but with relatively normal differentiation. The tumour mucosa is normally built around a core of arborising muscle extending from the stalk base to the tumour fonda [30].

In terms of the cellular mechanism driving polyp formation, it has been suggested that this occurs due to an expansion of the progenitor cell compartment and a disruption of a balance between symmetrical and asymmetrical stem cell division leading to mucosal prolapse [30,31]. Indeed, polyps may actually reflect mucosal prolapse rather than true hamartoma [30], with the polyps having relatively little malignant potential and PJS malignancy being driven out of overall mucosal instability rather than simple polyp progression. In this context, polyp removal may not lead to the reduction in the risk of cancer [30,31].

Although the intestinal polyps appear benign, PJS patients are however at risk of developing malignant tumours after the age of 30, and by the age of 65 the frequency of malignant transformation is 93% [27,32]. The most common PJS associated cancers are gastrointestinal in origin: gastric, small intestinal, colorectal, pancreatic and sometimes esophageal [28,29]. The risk of breast cancer in women is also elevated, being 8% at age 40 and it reaching 45% at the age of 70 [27]. Tumours can also develop in the ovary, lung, uterus, cervix, testis, sex cord and in Sertoli cells [28,33–40].

2.2. Somatic inactivation of LKB1 and human tumourigenesis

PJS can develop not only as a result of germ-line inactivation, but also can arise from somatic mutations [41]. However, LKB1 mutation has not been yet been reported as a frequent phenomenon in colorectal cancer [42]. Screening for somatic mutational inactivation of LKB1 in human breast and testicular cancers also failed to reveal high frequencies of mutation [42–44], although analyses of sporadic pancreatic and biliary adenocarcinomas has shown some elevation in mutation frequency, with 4–6% of them being associated with LKB1 sporadic mutation [45].

LKB1 has recently been shown to be frequently mutated in lung cancer (>30% [46,47]) suggesting that the mutation frequency in the original study [43] may have been underestimated. Indeed, more sensitive methods of detecting LKB1 mutations, such as the use of multiplex ligation-dependent probe amplification (MLPA), have revealed higher mutation frequencies than previously reported. Thus, the LKB1 mutation frequency in cervical cancer has recently been reported to be as high as 20%, considerably elevated compared to previous estimations. Furthermore, these studies have suggested that LKB1 mutation is associated with accelerated disease progression [19]. The authors of this report also found that the majority cervical cancer cell lines, including HeLa, contain Lkb1 biallelic mutations [19]. The absence of LKB1 protein in HeLa cells had been previously reported [48] but it was attributed to promoter methylation rather than mutation [49].

Despite the situation in HeLa cells, epigenetic suppression of LKB1 does appear to be an important mechanism of LKB1 inactivation, with LKB1 promoter hypermethylation present in 45% of papillary breast carcinomas and 18% of hamartomatous polyp lesions [49]. These observations suggest that the rate of epigenetic suppression may be higher than thought in other tumour types. For example, downregulation of LKB1 expression in endometrial cancer is significantly more common according to tissue microarray than would be predicted from mutation frequency, implicating epigenetic suppression [50].

2.3. Lung cancer

The most common cause of tumour-related death worldwide is lung cancer. It is also the most actively studied cancer in relation to LKB1 inactivation apart from PJS. When chromosomal alterations were analyzed in lung tumours, a 19p allelic imbalance was identified as a frequent event in tobacco-exposed patients [51]. Subsequent genetic analysis of primary lung adenocarcinoma samples revealed that a third of them harboured LKB1 mutations, and that all of these were somatic [18]. These findings were further supported when a large number of lung cancer cell lines were screened for both genetic alterations and changes in LKB1 expression [46,47,52,53]. LKB1 deficiency was shown to be particularly common in non-small cell lung cancers, (NSCLCs) and primarily affected males and smokers. LKB1 mutations were also found to be significantly more frequent in poorly differentiated adenocarcinomas than in those with higher degrees of differentiation [46,52].

Another characteristic feature of LKB1-deficient human lung cancers is that they have been observed concurrently with KRAS mutations [18,46]. Further evidence of functionality for K-Ras in mediating tumourigenesis comes from the observation that tumours characterized by concomitant LKB1 and K-Ras mutations exhibit increased sensitivity to both MAPK and mTOR inhibition [54]. Although male PJS patients do show elevated levels of lung cancer compared with the general population, this incidence is not as high as for other types of PJS-associated cancer [27,28], suggesting that multiple events including K-Ras activation are necessary to drive LKB1-associated lung tumourigenesis. This requirement for other interacting mutations is further underlined by the genetic variability observed between Caucasian and Asian patients, with the LKB1 mutation rate being far higher in NSCLC tumours and cell lines derived from the patients of Caucasian origin then in the patients of Asian origin [46,52,55,56].

3. Animal models of LKB1 loss

The importance of LKB1 in human disease was originally highlighted when its loss of function was linked with PJS development [17]. Disease suppression by LKB1 has now been studied in a range of different tumour types as outlined above and also in other disease settings, such as metabolic syndrome and type II diabetes [57–59]. To better understand the relationship between LKB1 and these different pathologies, a range of model organisms have been utilized including yeast, worms, Drosophila, frogs and mice [60–67]. It is becoming obvious that the role played by LKB1 is complex, impacting upon a wide range of signalling pathways and producing highly tissue-specific effects. Thus, different models of Lkb1-deficient cancer implicate different mechanisms. For example, gastrointestinal polyposis in Lkb1-deficient mice affects both the mTOR and TGFβ pathways [68–69], whilst in contrast endometrial cancer shows AMPK downregulation without overt changes in mTOR [51]. In terms of pathway interactions there are also very clear differences, such as p21/p53 pathway downregulation in pancreatic cancer driven by Lkb1 haploinsufficiency [70], the interaction with Src/Fak in lung cancer [71] compared to the involvement of the Rb pathway in skin cancer [72].
3.1. Gastrointestinal polyposis following Lkb1 loss and the interplay between mTOR and TGFβ

Whereas constitutive Lkb1−/− knockout mice are embryonic lethal [64], heterozygous Lkb1+/− mice created in different laboratories have been shown to be viable and prone to severe polyposis at around 8 months of age [65–67]. Gastrointestinal hamartomas of Lkb1 heterozygous mice share similar histological features compared to human Peutz–Jeghers polyposis and it has also been demonstrated that biallelic Lkb1 inactivation is not necessary for polyp development. [65–67,73,74]. At the same time, histological examination of polyps failed to find dysplastic or adenomatous changes suggesting that the polyps themselves do not progress [66], which is again reflective of the human situation.

One of the most important unresolved issues is the precise mechanism that drives Lkb1-deficient polyp development. mTOR pathway deregulation is an obvious candidate since Lkb1 negatively regulates mTOR via AMPK/TSC2 [75,76]. Consistent with this, Lkb1-deficient tumours show hyperactivation of mTORC1, with rapamycin treatment dramatically suppressing preexisting polyps and also downregulating hypoxia-induced factor HIF-1α and its targets [69,75]. Furthermore, activators of AMPK such as metformin, phenformin or A-769662 have all been shown to significantly delay tumour onset in mice with reduced levels of Lkb1 and PTEN, again mechanistically implicating mTOR [77].

Alternatively, loss of TGFβ-mediated communication between smooth muscle cells and the epithelium may drive intestinal disease [68]. Defects in mesenchyme-originated TGFβ production leading to epithelial alterations have been documented before [78], and the control of TGFβ production by Lkb1 may also explain its tumour suppressor function. Thus, when Lkb1 was conditionally removed from smooth muscle cells using Tagln (SM22) driven Cre recombinase, the mice developed multiple gastrointestinal tumours [68] and the resulting phenotype was reminiscent of constitutive Lkb1 heterozygous mice [65–67]. Lkb1 deficiency targeted to the mesenchyme resulted in a drop in TGFβ production and subsequent decrease in phospho-SMAD2 in the epithelium [68]. The resulting tumours showed hyperplastic epithelium and a strong stromal component and smooth muscle core (identical to the polyps arising in Lkb1−/− mice). These results argue for a primary role for the mesenchymal compartment in this mouse model of Peutz–Jeghers syndrome (PJS) [68].

The exact mechanism by which Lkb1 regulates TGFβ production remains unknown, but it is plausible to speculate that it may arise as a consequence of abnormal cell polarity and differentiation leading to defects in TGFβ ligand secretion and maturation. For example, Lkb1 deficiency causes defective myogenic differentiation with decreased smooth muscle actin expression and defective actin filament [79]. Furthermore, several Lkb1-deficient models have shown defects in cytoskeleton and polarity [7,80], phenotypes which may well also underlie perturbation of TGFβ production.

The link between the PI3K/Akt/mTOR pathway and TGFβ signalling is particularly interesting given that defects in either pathway may lead to similar hamartomatous polyposis. Thus, PJS, Tuberous Sclerosis Complex and Cowden Syndrome are respectively caused by deficiencies in LKB1, TSC1/TSC2 and PTEN [17,81,82]. Given the above discussion of the role of TGFβ in polyposis, it is also notable that Juvenile Polyposis is largely associated with SMAD4 loss [83]. It is however not yet clear whether misregulation of the mTOR and TGFβ pathways represents completely separate events or if they are affecting each other in some way. For example, it appears that Lkb1-mediated TGFβ ligand secretion is not controlled by mTOR, since the loss of TGFβ production in Lkb1-deficient cells was not reverted by rapamycin treatment, nor were overt changes observed in either AMPK or mTOR expression [68]. At the same time, it would be still interesting to assess the effects of rapamycin on tumour development in the SM22-Cre model. By contrast, the PI3K/Akt/mTOR has been implicated in events downstream of the TGFβ receptor. Thus, TSC2 can interact with the SMAD proteins and colocalize to the nucleus following stimulation by TGFβ and this cooperation can suppress cell proliferation by activating both p21 and p27 expression [84]. This is an area that clearly needs further examination, especially as the appropriate genetic tools are now available to probe this interaction.

3.2. Lkb1 deletion disrupts cell polarity, cell adhesion and can lead to abnormal differentiation and metastasis

Lkb1 is a key player in establishing the correct polarity in C. elegans [15,85] and in Drosophila [62,86]. Lkb1 mutations in Drosophila abrogate proper localisation of proteins and affect spindle formation [87]. In mammalian models, Lkb1 loss often leads to combined defects in cell adhesion, polarity and motility creating a potential for tumourigenesis and metastasis [71,88].

Lung cancer is the tumour type in which the role of Lkb1 in migration, adhesion and polarity is perhaps been most intensively studied, given the clear importance of Lkb1 somatic mutations in human lung [18,89]. Given the coincidence of Lkb1 mutations and K-Ras mutations in human tumourigenesis mentioned above, this potential interaction has now been modelled in the mouse, with combined mutation resulting in a much stronger synergistic effect than concomitant mutations of K-Ras with other tumour suppressors such as p53, Ink4a/Arf or p16 [57]. Thus, mouse survival was greatly reduced, with animals developing lung tumours belonging to a broad histological spectrum and with a high ability to invade and metastasise [57]. The same research group also concentrated on expression and phosphoproteomic changes occurring in these K-Ras mutant tumours with or without Lkb1 as well as with or without metastasis [71]. They found several signatures specific for epithelial-mesenchymal transition such as Snai1, Twist1 and also SRC and FAK activation suggesting increased cellular migration as well as impaired cell adhesion [71]. The authors also showed that Lkb1 loss can lead to an addiction to SRC and FAK signalling in both cell migration and adhesion. Thus in this setting Lkb1 deletion not only relieves mTOR signalling but also activates the SRC/FAK axis, so linking metastasis with tumour cell growth [71].

Recent studies have suggested that Lkb1 may affect cell motility via several different mechanisms. The LKB1/AMPK axis has been shown to regulate tight junction assembly and cell polarity [90,91], it is able to suppress tubulin polymerisation via activating microtubule-associated protein (MARK) signalling [92], and it can also phosphorylate PAK1, a key protein controlling cell motility [93]. The interaction of LKB1 with PAK1 is crucial to cell polarization and motility, with co-localization of LKB1 and PAK1 at the actin scaffold leading of the edge of a cell leading to Lkb1-mediated phosphorylation of PAK1 and subsequent recruitment and activation of cdc42. Normal functioning of these processes appear to be required for cell polarity events, such as lamellipodia formation and Golgi reorientation, with LKB1 appearing to be a dynamic, actin-associated protein, the depletion of which results in classic cell polarity defects, such as aberrant morphology and organelle positioning [88].

Interesting results were obtained using 3D organotypic mammary acinar structures [94]. Mammary epithelial cells can develop poorly differentiated cysts if cultured in collagen matrix but they are able to form polarized epithelial structures if cultured in Matrigel instead. This organized epithelial structure can prevent proliferation caused by c-Myc activation. When the tissue architecture and polarity was disrupted by LKB1 loss, c-Myc driven proliferation in acinar structures was reinitiated. The disruption was mTOR-independent and rapamycin did not inhibit the reinitiation of the cell cycle by c-Myc [94].
Another interesting aspect of Lkb1 biology is its role in regulating anoikis. Cell that lose anchorage to their native matrix often undergo this detachment-induced cell death [95], with clear implications for this process in tumour invasion and metastasis. LKB1 has been shown to participate in this process via the phosphorylation of SIK1 kinase, a role which has been demonstrated to be important in the p53-dependent suppression of anchorage-independent growth [95].

Lkb1 deficiency has also been shown to cause significant polarity defects in the pancreas, mostly attributed to inactivation of the downstream AMPK/MARK/SAD family kinases. These defects included cytoskeletal abnormalities, loss of acinar cell polarity and tight junctions, which are considered to contribute to the development of pancreatic serous cystadenomas, a tumour type associated with PJS [7]. The interaction of Lkb1 with Par1b/MARK2 also seems to be crucial in maintaining beta cell polarity and intracellular cilia positioning as evidenced from the phenotype of pancreaticconditional deletion using Pdx1-Cre [96].

The role of LKB1 in polarity has been reported to be crucial in axon initiation and differentiation in cultured hippocampal neurons [97]. In this context, the LKB1/STRAD pair mediates the development of undifferentiated neuritis, with local accumulation of LKB1/STRAD considered to bias growth capacity towards one neurite and that this process can promote axon initiation [97]. These findings were complimented when LKB1 expression was selectively disrupted in the murine neocortex. This prevented axon formation without changes in neuronal migration and cortical layering. The authors proposed that in this setting Lkb1, together with downstream SAD-A and SAD-B kinases, is indispensable for axon formation in the neocortex [6]. Similarly, Lkb1 has been reported to specify axon and dendrite identity, as deletion of Lkb1 in migrating immature neurons disrupted centrosome positioning as well as axonal and dendritic polarity, ultimately resulting in impaired neuronal migration and differentiation [98].

We have recently shown that loss of Lkb1 can also affect intestinal differentiation. Selective removal of Lkb1 from the mouse intestinal epithelium leads to the expansion of secretory cell lineages together with changes in their differentiation [99]. This expanded population retains features of both Goblet and Paneth cells, the two major secretory cell types within the intestine, suggesting a failure of normal differentiation of these lineages. This phenotype is characterized by deregulated Notch signalling, possibly due to reduced Delta ligand levels in the secretory cells, which may in turn be due to greatly diminished MARK kinase phosphorylation [99].

Defects in cell polarity, adhesion and function have been in a spectrum of other Lkb1-deficient tissues. For example, we have also shown that Lkb1 deletion in the prostate leads to loss of tight junctions and initiates tumourigenesis [100]. Furthermore, others have shown that the Lkb1/AMPK axis is important in hepatocyte polarity and canalicular network formation in bile ducts [101].

3.3. Lkb1 controls mouse angiogenesis

Constitutive homozygous ablation of Lkb1 causes lethality at embryonic day 11 [64]. Lkb1 null embryos several angiogenic phenotypes, including thin and discontinuous aorta, rudimentary yolk sac vasculature and little or no invasion of allantoic blood vessels into the placenta [64]. Similar results were obtained when Lkb1 was deleted conditionally using Mox2-Cre recombinase which spared Lkb1 in trophoblasts but caused deletion in the embryo, allantois and yolk sac mesoderm [102]. To determine whether lethality arises as a consequence of a cell-specific role of Lkb1 in the vascular system, mice have been generated using the Tie1-Cre transgene to drive endothelium-specific Lkb1 deletion [102]. In this model, deletion of Lkb1 in the endothelial lining of blood vessels reduced TGFβ production and resulted in fragile and distorted vessels characterized by loss of the supporting vascular smooth muscle cells necessary for stabilization and maturation. This phenotype therefore largely mimicked that seen in the constitutive knockouts [64]. It was also similar to the phenotypes observed following TGFβ pathway deregulation, such as that seen after smooth muscle specific deletion of TGFβRII [103]. Consistent with these similarities, Lkb1-deficient embryos showed a clear downregulation of the TGFβ pathway, with levels of phosphorylated SMAD2 significantly lower both in Lkb1-deficient yolk sac and in mesenchymal cells [102]. These observations suggest that the endothelial cells are relying on TGFβ-based communication between endothelium and smooth muscle to recruit smooth muscle cells into the newly formed blood vessels. The role of Lkb1 in this process appears to be in regulating TGFβ availability to the nearby smooth muscle and mesenchymal cells, such that deficiency of Lkb1 results in the incomplete coverage of maturing vessels with smooth muscle cells [102].

Lkb1 has also been studied in relation to the revascularization response to ischemia [104]. The approach used here was to use Tie2-Cre driven deletion of Lkb1, again within endothelium. Homozygous deletion resulted in embryonic lethality, however heterozygous deletion resulted in normal capillary density if unchallenged, but a greatly reduced response to ischemia when assessed at the microcirculatory level. In terms of mechanism, these effects appear to be mediated through the interaction with AMPK, with the speculation that stress, nutritional deprivation and hypoxia activate AMPK signalling in endothelial cells, such that the Lkb1-AMPK axis then stimulates a pro-angiogenic response.

3.4. Lkb1 and AMPK influence feeding behavior, adiposity and nutrient rationing

Food shortages or stressful environmental conditions often affect animal development, metabolism and behavior. In mammals, the hypothalamus plays a key role in appetite control and subsequent behavioral strategy by integrating satiety signals coming from both adipose tissue and the gastrointestinal tract [105]. Both AMPK and Lkb1 play prominent roles in the hypothalamus, influencing feeding behavior and metabolism in response to these signals [106]. In support of this, Lkb1 deletion using the RIP2.Cre transgene, which is active in both pancreas and hypothalamus, has been shown to reduce food intake and weight gain [107]. Similarly, activation of AMPK in rats can increase food intake [108], and AMPKβ1-deficient mice are characterized by both reduced body weight and appetite [109]. However, this regulation is clearly complex, as constitutive knockouts of either AMPKα1 or AMPKα2 show no change in body weight or appetite [110]. Furthermore, AMPKα2 deletion in different types of neurons has been shown to lead to opposing effects on body weight, possibly reflecting differences between hormone sensing cells and glucose sensing ones [111].

Clearly, Lkb1/AMPK signalling is important in feeding/satiety and this may have potential ramifications for obesity research. In this context, the interaction of Lkb1 with Fyn tyrosine kinase is of particular interest, as mice bearing a knockout of Fyn tyrosine kinase have been reported to be leaner with increased activation of AMPK in the adipose tissue [112]. Given that Fyn phosphorylates Lkb1 at tyrosine residues 261 and 365 with the consequence that Lkb1 is retained in the nucleus, then inhibition of Fyn should lead to Lkb1 cytoplasmic export and subsequent activation of AMPK-driven energy expenditure with the ultimate result of a reduction in fat reserves [113].

In contrast to mice, the Lkb1/AMPK pair in the nematode C. elegans actually protects its energy reserves during the dauer stage [61]. C. elegans is known to enter dauer diapause in harsh
environments. Before entering this phase they feed and build fat and carbohydrate reserves. After entering diapause they stop feeding and ration their nutrient storage, with a resultant increased lifespan [114]. AMPK appears a critical regulator of this process, as deletion of the α2 catalytic subunit of AMPK leads to more than a threefold reduction in animal survival. Consistent with this, double knockouts of the β1 and β2 subunits of AMPK as well as of the Lkb1/STRAD pair both result in similar decreases in longevity [61].

3.5. Lkb1 loss in liver affects whole body glucose metabolism in a diametrically opposed fashion to the loss of pancreatic Lkb1

Type 2 diabetes is commonly associated with high blood glucose combined with insulin resistance. High blood glucose is usually the result of excessive glucose production in the liver (gluconeogenesis). Lkb1 has been implicated in these processes as liver-specific deletion leads to increase in both gluconeogenesis and lipogenesis, both of which are significant energy consuming processes [115]. The mechanism underlying this role appears to be the phosphorylation of CREB-regulated transcription coactivator 2 (CRT2 or TORC2) by Lkb1/AMPK. The consequence of such phosphorylation is relocation to the cytoplasm and consequent prevention of gluconeogenic program induction [115].

Metformin is the key anti-gluconeogenic drug used for the treatment of diabetes, and it has been suggested that the Lkb1/AMPK axis is the main executor of its action [115]. However, it has recently been shown that metformin can also suppress gluconeogenesis directly, bypassing Lkb1/AMPK [116]. Metformin actions may indeed be wider that AMPK activation as the principal function of the drug is to inhibit ATP production by complex I of mitochondrial oxidative phosphorylation, so creating a AMP/ATP ratio, which in turn not only acts on the Lkb1/AMPK axis but also directly affects gluconeogenesis and glycolysis via the allosteric inhibition of key enzymes [117].

Remarkably, whereas liver-specific Lkb1 deletion leads to high blood glucose levels due to unrestricted gluconeogenesis, the deletion of Lkb1 in the pancreas actually enhances glycemic control [96,101,107]. Thus, mice deficient for pancreatic Lkb1 (Pdx1-Cre) had normal fed and fasting glucose levels but they displayed a faster and more efficient response to glucose than wild-type animals [96,101,107]. The mechanism underpinning this remains largely unclear, but may simply reflect elevated serum insulin levels as a consequence of enlarged pancreatic β-cells in the absence of Lkb1 [96,101,107].

3.6. Lkb1 loss and cell survival

The role of Lkb1 loss in the regulation of apoptosis appears to be highly tissue specific. Thus, depending on a cell type, Lkb1 loss can either compromise apoptosis or enhance it. The pro-apoptotic role of Lkb1, which might be considered consistent with its role as a tumour suppressor, has been relatively well described. Thus, Lkb1 has been shown to be involved in TRAIL-mediated apoptosis in osteosarcomas [118]. As already discussed it controls p53-dependent anokis [95] as well as p53-dependent apoptosis [119], the latter via translocating Lkb1 into the mitochondria [119].

At the same time Lkb1 deletion can compromise survival of those cells which have tight energy rationing, such as lymphocytes. The normal immune response often requires bursts in lymphocyte proliferation and this is obviously an energy demanding process. Given the role of Lkb1 in regulating energy production/demands, Lkb1 status would be predicted to be critical to this tissue type. Recently, two separate groups have described Lkb1 loss in T-cells. They both used Cre-recombinase with expression driven by the lymphocyte protein tyrosine kinase (Lck) promoter to generate LckCreLkb1<0D>10<0D> mice [120–122]. Although the mice were viable, they had reduced numbers of thymocytes, substantial amounts of T-cell death and very small thymi with abnormal tissue architecture [120,121]. Analysis of the T-cell populations in these mice revealed a reduction in double positive mature thymocytes (with both CD4+ and CD8+), but higher levels of immature double negative T-cell progenitors, which were largely arrested at the CD4+ CD8+ CD44+ CD25<0D>(DN3) stage. As described above, T-cell survival was compromised by deficiency of Lkb1, however the cells could be rescued by either ectopic expression of Bcl-XL or constitutively active AMPK expression, again underscoring the anti-apoptotic role of the Lkb1/AMPK pair in this setting [120].

One view of the Lkb1/AMPK pair is that they function as part a cellular mechanism preventing energy addicted cells from entering apoptosis triggered by an elevated AMP/ATP ratio. Indeed, mimicking high AMP/ATP ratios by AICAR treatment can induce apoptosis in lymphocytes, although neither phenformin nor the direct AMPK activator A-769662 have the same effect [123]. Moreover, metformin, a drug known to activate AMPK/Lkb1 [114] can also promote mature T-cell survival [124]. It is tempting to suggest that in energy-rich cells, Lkb1 loss permits higher proliferation rates and ultimately tumourigenesis, however such a mechanism would drive the expansion of cells which would be much more vulnerable to death in a context of failing ATP levels due to Lkb1 dysfunction. It is perhaps more tempting to speculate that Lkb1 can allow adaptation to metabolic stress [125] and its deletion may selectively eliminate some vulnerable cell lineages in favour of more stress resistant ones.

3.7. Lkb1 deficiency causes cardiac hypertrophy and affects skeletal muscle physiology

Cardiac tissue is capable of adaptive growth in response to various different stimuli including pregnancy or exercise [126]. This adaptation is underpinned by increased protein synthesis, with mTOR playing a key role in this process [127]. As in other tissues, Lkb1 has been suggested to regulate mTOR [128]. To specifically examine its role in this tissue, mice have been generated with a cardiac-specific Lkb1 deletion driven by a αMHC-Cre transgene. These mice exhibited severe atrial and ventricular hypertrophy, fibrillation changes and increased mortality [129]. The hypertrophic effect in these phenotypes appears to be due to abnormal mTOR activity, as shown through in vitro experiments with the mTOR inhibitor rapamycin and constitutively active AMPK [129]. Notably, regulation of protein synthesis through mTOR is not the only cardiac cellular mechanism influenced by Lkb1, as the Lkb1/AMPK pair is also responsible for the increase in long chain fatty acids and glucose uptake (the main fuel for cardiac ATP production) in response to contraction or exposure to ATP/AMP ratio lowering agents such as AICAR [130].

A slightly different phenotype has been reported from mice in which Lkb1 has been deleted in both cardiac and skeletal muscle using a muscle creatine kinase promoter driven Cre (MCK-Cre). Although these mice did not develop a severe cardiac phenotype, cardiac function was again impaired and mTOR/p70S6 signalling increased due to impaired AMPK phosphorylation [131,132]. Taken together, these studies support a potent role for Lkb1 in cardiac muscle regulation, although there are clear differences in phenotype which may reflect cell type specificy or simply the extent of recombination delivered by the different cre transgenes. Somewhat similar phenotypes have also been observed in Lkb1 deficient skeletal muscle, with reduced AMPK activation and an increased AMP/ATP ratio [133]. Although Lkb1-deficient muscle showed increases in glucose uptake and improved glucose tolerance [134], they had a deficient contraction-stimulated glucose uptake [133] as well as decreased voluntary running ability [135].
4. Conclusions

Lkb1 was initially discovered because of its role as a tumour suppressor and it is mutated in a number of cancers (PJS, lung and cervical cancer) but it is becoming apparent that Lkb1 has a wide range of biological roles that reach far beyond that. This serine-threonine kinase serves as a master kinase and its loss can affect a variety of biological processes including energy metabolism, cell structure, migration, adhesion and polarity (Fig. 1). Recent “in vivo” data obtained on animal models suggested that Lkb1 is often responsible for coupling cell growth, structure and survival to nutrient conditions and energy rationing. Lkb1 deletion can selectively eliminate some cell types and favour proliferation of others. In vivo effects of Lkb1 loss seem to be highly complex and they depend on a number of other biochemical settings. We cannot underestimate the importance of animal models providing us with an excellent opportunity to study tissue-specific Lkb1 loss and find better therapeutic approaches for a variety of pathological conditions.

References


