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Review

Molecular mechanisms of tumor suppression by LKB1

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ABSTRACT

The *LKB1* tumor suppressor gene is frequently mutated in sporadic lung adenocarcinomas and cervical cancers and germline mutations are causative for Peutz-Jeghers syndrome characterized by gastrointestinal polyposis. The intracellular LKB1 kinase is implicated in regulating polarity, metabolism, cell differentiation, and proliferation – all functions potentially contributing to tumor suppression. LKB1 acts as an activating kinase of at least 14 kinases mediating LKB1 functions in a complex signaling network with partial overlaps. Regulation of the LKB1 signaling network is highly context dependent, and spatially organized in various cellular compartments. Also the mechanisms by which LKB1 activity suppresses tumorigenesis is context dependent, where recent observations are providing hints on the molecular mechanisms involved.

1. Genetic evidence linking LKB1 to tumor suppression

Searches for the causative mutations of Peutz-Jeghers syndrome (PJS) identified the serine threonine kinase *LKB1* as the culprit in 1998 [1,2]. The most prominent symptom associated with PJS is gastrointestinal polyposis, typically manifesting during the second or third decade of life and is diagnosed by abdominal pain, bleeding or endoscopy/colonoscopy. The earliest symptom often is mucocutaneous pigmentation around the lips, oral mucosa, face, genitalia or palmar surfaces [3]. Benign polyps arise throughout the gastrointestinal tract, and are pedunculated in shape and classified as hamartomas [3]. Although hamartomas are expected to represent most of the differentiated cell types found in the adjacent normal tissue, recent studies suggest that the PJS polyps display differentiation defects both in the epithelial and stromal components with an increase in myofibroblasts [4,5]. Current treatment of PJS patients is regular screening for polyps and surgical interventions as a response to an acute occlusion but also to prevent gastrointestinal tract occlusions [6].

Currently it appears likely that a germline mutations of *LKB1* underlies all properly diagnosed PJS as different approaches have identified *LKB1* mutations almost in all PJS-patients [6]. The causal relationship is independently demonstrated by the remarkably similar polyposis in mice carrying germline inactivating *Lkb1* mutations [6]. Interestingly, apparently identical polyps also occur in mice where *Lkb1* is inactivated only in stromal SM22-expressing cells of the smooth muscle lineage implicating the prominent smooth muscle component in PJS polyps as an important driver of polyposis [4].

Epidemiological studies have implicated 41–60% overall risk for PJS patients of developing first cancer at the age of 60, whereas general population risk at this age is 8.5% [7,8]. Accordingly, *Lkb1* heterozygote mice in addition to the fully penetrant polyposis occasionally develop liver carcinomas [9], endometrial cancer [10] and osteogenic tumors [11]. *LKB1* mutations have also been identified in sporadic tumors, but in contrast to the enrichment of gastrointestinal cancers in PJS patients [7], sporadic mutations have been commonly identified from non-small cell lung carcinomas (NSCLC) [12,13–15] and cancers of the uterine cervix [16]. In NSCLC mutations are more frequent in adenocarcinomas [13,14,17]. Biallelic inactivation of LKB1 has been indicated in cervical cancers, whereas one wild type allele is retained in lung adenocarcinomas (see below) [12,13,16]. As several cancer mutation identification methods have relied on loss of heterozygosity (LOH) it is plausible that current cancer genome sequencing efforts will identify new tumor types with LKB1 mutations. However, cancer genome screens so far have not identified significant amounts of LKB1 mutations in tumor types studied [17,18].

There is considerable variability (0–34%) in reported *LKB1* mutation frequencies in lung adenocarcinomas [12–15,17–20], probably partly due to methodology, but also likely reflecting differences in environmental variables and genetic background of studied patients. Indeed, *LKB1* mutations are common in tumors

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of Caucasian smokers [14] and absent in tumors of Asian non-smokers [19]. Inactivating *LKB1* and activating *EGFR* mutations are largely mutually exclusive [14,20], and EGFR mutations in turn are common in the Asian population [12]. The exclusivity can be due to (i) EGFR activating and LKB1 inactivating a common cancer-promoting pathway, (ii) increased sensitivity of cells carrying *LKB1* mutations to oncogenic stress by EGFR or (iii) EGFR tumors and LKB1 tumors represent separate subtypes of lung adenocarcinomas with differing "addictions" for mutations. *p53* (*TP53*) and *KRAS* mutations overlap with *LKB1* mutations [13,20] and the overlap between *LKB1* and *KRAS* in one study indicates cosegregation [14]. In a mouse lung cancer model driven by *KRAS* mutations inactivation of *Lkb1* increased tumorigenicity more than p53 mutations consistent with the notion that LKB1 and KRAS cooperate in lung tumorigenesis [13].

In cancers of the uterine cervix the only recurrent mutation identified is in *LKB1*, identified in up to 20% of cervical cancers, including the tumor used to generate the HeLa cell line [16]. This warrants a closer look at the potential cooperation between LKB1 and the known factors promoting cervical cancer including the E6 and E7 oncogenes of the Human papilloma virus (HPV)-genome present in almost all cervical cancers. HPV E6 and E7 inactivate p53 and retinoblastoma (pRB) functions [21], and a further promoter of cervical cancer is estrogen expression [22]. Here studies on *Lkb1* inactivation in *E6* and *E7* expressing mouse models of cervical cancer [22] might reveal more detailed mechanisms on how *LKB1* activity intersects with these pathways. The cooperation between estrogen and *LKB1* is supported by studies in endometrial cancer, where an inverse correlation between *LKB1* staining and endometrial cancer grade was noted in human tumors [10], and where *Lkb1* mutations promote tumors in mice [10,23].

2. Haploinsufficiency and biallelic inactivation of *LKB1*

A meta-analysis of reported *LKB1* mutations [24–27] in Peutz-Jeghers Syndrome indicates that 60% ($n = 92$) of polyps retain a wild type allele, suggesting that biallelic inactivation as detected by loss of heterozygosity is not a required event for tumorigenesis. Mice carrying germline inactivating mutations of *Lkb1* phenocopy PJS polyposis with full penetrance [6] and thus allow for a robust platform to analyze this and other molecular changes leading to polyposis. Biallelic inactivation was either not detected [28] or only noted in part of the tumors [29]. These results suggest that *Lkb1* is haploinsufficient in suppressing PJS tumor initiation consistently with its behavior in regulating signaling in cell culture [4,30]. At the same time the occasional identification of biallelic inactivations in both human and mouse polyps indicates that a "second hit" may provide a further growth advantage as noted in one study [4] and warrants further investigation.

Biallelic inactivation of *LKB1* as detected by LOH was not commonly identified in recent analyses of NSCLC [12,13]. Also the lung adenocarcinomas in *KRAS;Lkb1*$^{+/-}$ mice retain expression of wild type *Lkb1* [13]. *LKB1* mutations may be early events in NSCLC as mutation frequency does not correlate with clinical stage [14]. These observations suggest that carriers of germline *LKB1* mutations should be at high risk of NSCLC which has not been noted in PJS patients [7]. Possible explanations might be altered smoking behavior or decreased life expectancy of PJS patients. It is also possible that *LKB1* heterozygosity at a very early point in tumorigenesis is disadvantageous e.g. due to oncogenic stress where *LKB1* mutations in sporadic lung adenocarcinomas would take place only after genetic changes conferring resistance to oncogenic stress.

In *KRAS*-induced lung cancer in mice *Lkb1* inactivation increased not only tumor number but also tumor burden and metastases [13] and resistance to PI3K-mTOR and MEK pathway inhibition [31]. These phenotypes benefited from biallelic deletion [13] consistent with the notion that a "second hit" provides a further advantage for tumor progression. This would predict a higher frequency of LOH or epigenetic silencing of wild type *LKB1* allele in metastasis compared to primary tumors. Also the noted loss of *LKB1* expression in some lung adenocarcinomas [12,15,32] should correlate with worse disease outcome.

In contrast to NSCLC, *LKB1* mutations in cervical cancer represent biallelic inactivations. Interestingly, they also associate with poor prognosis [16]. The much less frequently noted *LKB1* mutations in other tumor types have mostly been biallelic e.g. in liver, colorectal, breast, pancreas and sex cord tumors [6]. Accordingly, in *Lkb1* heterozygote mice LOH was found in the more rare tumors of the liver [9], endometrium [10] and in carcinogen-induced skin cancer [33]. *LKB1* LOH has also been detected in 76% ($n = 29$) of PJS-patient carcinomas [24–27]. Thus biallelic inactivation of *LKB1* is more common in cancers than in PJS polyps again supporting the notion that biallelic inactivation promotes progression.

3. LKB1 kinase complex and its regulatory mechanisms

LKB1 encodes a serine threonine kinase of 433 amino acids, which is active in a complex with a pseudokinase STRAD (STRADα or STRADβ) and scaffold MO25 (MO25α or MO25β) [34,35]. Both kinase domain integrity [34] and in one case the ability to bind STRAD [36] appears to be crucial for LKB1-mediated tumor suppression. Mutations of *STRAD* or *MO25* genes have not been found in PJS patients [6] or adenocarcinomas of the lung [20], which may be due to redundancy or alternatively suggests LKB1 may act without STRAD and/or MO25 as recently identified for PAR-4, the *Caenorhabditis elegans* ortholog of LKB1 [37].

Early studies identified several substrates of LKB1 including p53 [38,39], PTEN [40], and LIP1 [41]. Attention was redirected with the identification of LKB1 as the activating kinase of the catalytic subunit of the AMP-activated protein kinase (AMPK) [42] as well as several other kinases with a T-loop activation domain similar to AMPK [43]. Together these form the identified 14 LKB1 substrate kinases: AMPKα1-2, BRSK1-2 (also known as SAD-B and SAD-A), MARK1-4, NUAK 1-2, SIK1-3 (SIK1, QIK and QSK, respectively) and SNRK [34]. The LKB1 kinase complex has been demonstrated to be required for the activation of these kinases in cell culture [42,43] and for several of them also *in vivo* [10,13,32,33,44–46]. Alternative activation mechanisms for the substrate kinases have only been identified for AMPKα1, which is phosphorylated and activated in heart and skeletal muscle in the absence of *Lkb1* probably by CamKK [34].

Regulation of LKB1 and the phosphorylation of the T-loop activation site of various LKB1 substrate kinases is highly cell type and context specific. In melanoma cells activated RAF signaling induces ERK and RSK to phosphorylate S325 and S428, respectively, which was suggested to compromise the ability of LKB1 to bind and activate AMPK [47]. In neurons, BDNF-induced activation of PKA in a single neurite outgrowth triggers localized phosphorylation of S428 (S431 in mice), which in this setting appears to stabilize the LKB1–STRAD–MO25 complex required for axon specification [44,48]. During immunoglobulin gene remodeling DNA strand breaks induce ATM, which in this context leads to phosphorylation of LKB1, inactivation of CRTC2 (also known as TORC2) and differentiation of B cells [49], which suggests a mechanism where ATM phosphorylation of LKB1 on T366 [50], induces LKB1 to phosphorylate either SIK or AMPK, both capable of phosphorylating and inactivating CRTC2 [51]. In myocytes and adipocytes Fyn-mediated tyrosine phosphorylation on Y261 and Y365 of LKB1 decreases cytoplasmic LKB1 and AMPK T-loop phosphorylation [52].

LKB1 has been identified in several subcellular compartments where in each it appears to mediate specific functions. The C-terminus of LKB1 contains a conserved prenylation site required for membrane localization and establishment of polarity in Drosophila oocytes [53] and for localization to adherence junctions in MDCK cells [54]. LKB1 in the primary cilium is implicated in cell size regulation through AMPK [55]. LKB1 has a functional nuclear localization signal [56,57] and chromatin-bound LKB1 has been implicated in activating AMPK leading to H2B-S36 phosphorylation and activation of p21 transcription in response to glucose deprivation (Fig. 1) [58]. On the other hand, LKB1 lacking the NLS retains the ability to restrict growth of LKB1-deficient cells, and STRAD and MO25 association enriches cytoplasmic LKB1 [36,59].

A further level of regulation is conformation of substrates. It has been proposed that T-loop phosphorylation of 10 out of 14 LKB1 substrates is enhanced by an intramolecular interaction between the kinase domain and a ubiquitin-associated (UBA) domain, where ubiquitinylation prevents this interaction [60,61]. The AMPK kinase complex contains regulatory AMPKβ and AMPKγ subunits. A myristoyl-switch of AMPKβ and AMP binding to AMPKγ have been suggested to stimulate LKB1-mediated T-loop phosphorylation of AMPKα [62], whereas other studies suggest that AMP binding to AMPKγ induces a conformational change in AMPKα making it a worse substrate for the phosphatase [63,64]. Spatial and temporal regulation of phosphatase activity is likely to be relevant for other LKB1 substrate kinases as well.

4. LKB1 and AMPK in control of proliferation and metabolism

LKB1 signaling has been implicated in promotion of proliferative senescence in a p53-independent manner [29,65] and of cell cycle arrest and cell death in p53-dependent manner [39,57,66–68]. p53-dependent regulation of cell number by LKB1 might be mediated by direct phosphorylation of p53 S15 by LKB1 substrate kinases AMPK and SIK1 in response to glucose starvation [67] or cell detachment (Fig. 1) [66]. p53 S15 phosphorylation induces p21 [69] and consistently LKB1-mediated cell cycle arrest of LKB1-deficient cells is p53-dependent and induces p21 levels [57]. These effects were mediated also by an LKB1 lacking the nuclear localization signal [57] suggesting that LKB1 was not involved in direct p53 S15 phosphorylation [39].

In considering the relevance of p53 and possibly S15 phosphorylation in LKB1 mediated tumor suppression an interesting comparison is offered by the ATM kinase strongly implicated in S15 phosphorylation. The observation that *p53* and *ATM* mutations are mutually exclusive in lung adenocarcinomas and mutation of either *p53* or *ATM* leads to increased mutation rates [20] suggests that p53 is critical for ATM tumor suppression. By contrast, *LKB1* and *p53* mutations are concurrent in lung adenocarcinomas [13,15,20] and *LKB1* mutations are not associated with increased mutations rates [20]. Also the tumor spectrum of *Lkb1* deficiency and *p53* deficiency in mice do not overlap, but *p53* mutations enhance polyp initiation in $Lkb1^{+/-}$ mice (our unpublished results) [70,71]. On the other hand, decreased LKB1staining in pancreatic cancers correlates with decreased p21 staining only in tumors that did not express mutant p53 [72]. These results suggest that decreased signaling from LKB1 to p53 may contribute to early stages of tumorigenesis, but that p53 loss in lung adenocarcinomas is a later event allowing increased mutation rates or tolerance to increased mutation burden of tumors.

In line with the ability of AMPK to induce cell cycle arrest, evidence for it playing a part in tumor suppression by LKB1 is emerg-

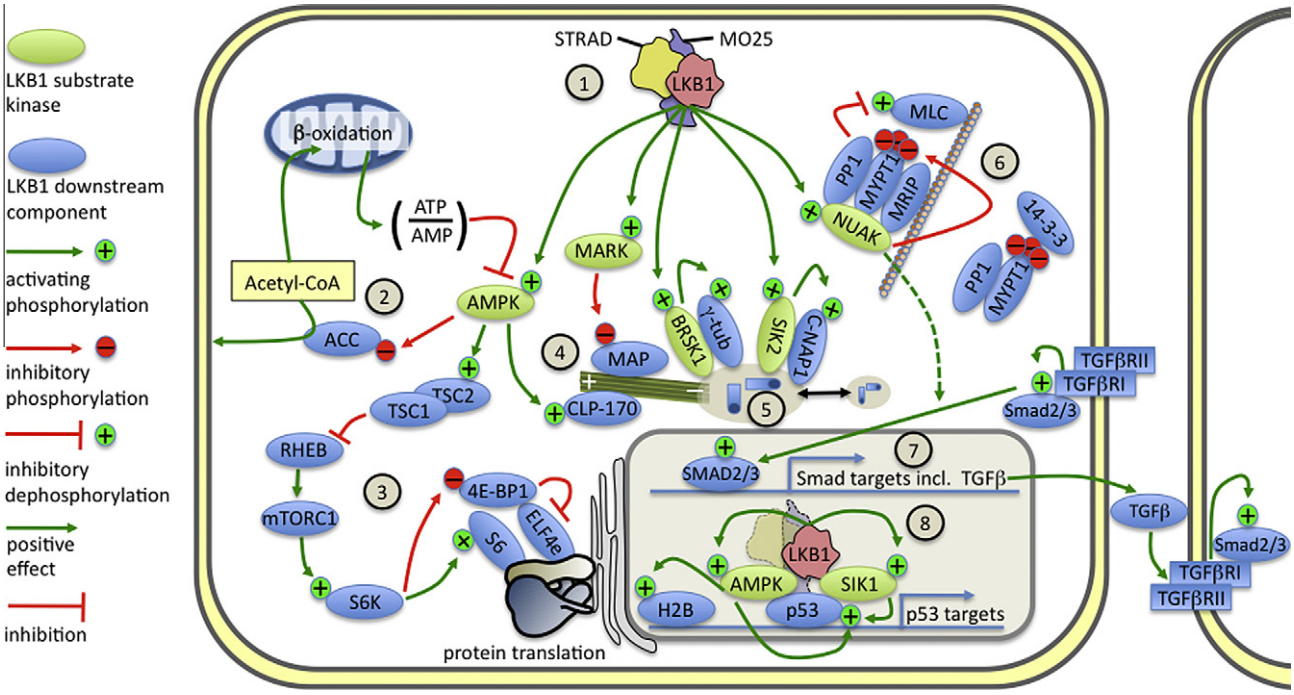

Fig. 1. Signaling by LKB1 substrate kinases potentially involved in tumor suppression. In mammalian cells LKB1 kinase complex (1), modified from [35] activates substrate kinases in several cellular compartments. Decreased energy levels lead to increased AMP which together with LKB1 activate the AMP-activated protein kinase (AMPK), which in turn inhibits ATP-consuming anabolic reactions such as fatty acid synthesis (2) and protein translation (3). AMPK phosphorylates CLIP-170 increasing microtubule stability, whereas MARK kinases phosphorylate microtubule associated proteins (MAPs) thus decreasing microtubule stability (4). The LKB1 substrates BRSK1 and SIK2 (5) are required for centrosome duplication and separation, respectively. NUAK kinases are involved in inhibition of MLC dephosphorylation (6), which can take place either via sequestration of the phosphatase complex (with PP1 and MYPT1 subunits shown) by 14-3-3 subsequent to phosphorylation of MYPT1 by NUAK or via MRIP-dependent inhibition of MLC phosphatase on actin fibers in partially kinase independent manner. In primary stromal cells LKB1 positively regulates TGFβ signaling and production (7), which may be mediated through NUAK and actin fibers (dashed arrow). In the nucleus LKB1 has been identified in a complex with AMPK and p53 and implicated in p53-dependent transcription via AMPK and SIK1; both phosphorylation of p53 and histone 2B (H2B) have been proposed as mechanisms. The involvement of STRAD and MO25 in these LKB1 functions have not been characterized (8). For references, please see text.

ing. Several epidemiological studies on type 2 diabetes patients have revealed a significant decrease in cancer incidence and mortality among patients treated with the AMPK activator metformin in comparison to non-treated, insulin or sulfonylurea treated patients [73,74]. Activation of AMPK with metformin, phenformin or A-769662 also led to decreased tumor burden in PTEN heterozygote mice, whereas *LKB1* hypomorphism and associated deficient activation of AMPK increased tumor burden of PTEN heterozygote mice [75]. Subsequent to these reports a wealth of clinical studies using metformin for treatment of diabetic and non-diabetic patients have been started (12 ongoing and 1 completed clinical trial can be found in http://www.clinicaltrials.gov).

Signaling downstream of AMPK is dedicated to improve cellular energetic state [63], whereas cancer cells are often using all possible energy to growth. Thus inactivation of AMPK signaling may be in the interest of cancer cells tilting the balance toward lipid synthesis and maintenance of mTOR activity, which stimulates protein translation via S6K and 4EBP1 (Fig. 1) [51] and possibly mediates AMPK-dependent autophagy [76,77].

On the other hand, also cancer cells can run into an energy deficit, and this can be exacerbated by the hypoxic environment and/or shift in energy production from mitochondrial citric acid cycle to glycolysis, known as the Warburg effect [78]. Consistently there are also tumor models where AMPK is activated [79,80], and could represent cells which have been initially glucose-addicted, but now experiencing low glucose [81] or where autophagy and fatty acid beta oxidation are otherwise critical [82]. Considering these variations use of AMPK activating drugs as cure for cancer should be carefully considered.

Although several lines of correlative evidence support a role for AMPK in LKB1 tumor suppression direct genetic evidence is scarce. Decreased AMPK activity has been noted in several *LKB1*-deficient mouse models [10,13,33,45]. Also mTOR pathway activation has been noted in epithelia of polyps in $Lkb1^{+/-}$ mice [83] suggested to be due to reduced AMPK activity. However, mTOR pathway was also activated in polyp epithelia without *Lkb1* mutations [4] indicating it is not linked with *Lkb1* mutations, and indeed mTOR pathway activation is very common in human tumors. Therefore attenuation of polyp growth with the mTOR inhibitor rapamycin [84,85] does not directly link AMPK to LKB1 tumor suppression in this model. In another study using the KRAS induced lung adenocarcinoma model the increased metastatic capacity of tumors with *Lkb1* mutations was suggested to be mediated through increased expression of lysyl oxidase (LOX) due to increased mTOR and HIF1α [86]. The apparent discrepancy between *LKB1* mutations (not correlated with clinical stage but correlated with smoking) [14] and *LOX* expression (correlated with clinical stage and metastatic status in human tumors but not with sex or smoking) [86] indicate further studies are needed to have direct evidence for a role for AMPK also in lung adenocarcinoma. Melanomas carrying *BRAFV600E* mutations have also been suggested to inactivate AMPK through LKB1 phosphorylation [47] as discussed above. The relevance for this pathway in melanoma is important to investigate further including analysis of how LKB1 phosphorylation affects LKB1 activity toward other substrates as well as how exogenous LKB1 arrests G361 melanoma cells carrying the V600E mutation (Cosmic database and) [36,57,87].

The lack of *AMPKα1* and *α2* mutations in PJS patients, sporadic lung adenocarcinomas, or other tumor types suggest that either *AMPKα1* or *α2* are not sole mediators of tumor suppression by *LKB1* in these tissues or alternatively that *AMPKα1* or *α2* are redundant in potential tumor suppressive function. Analysis of $AMPK\alpha1^{-/-};\alpha2^{-/-}$ mice indicate redundancy at least during embryogenesis [88]. However, the differential expression pattern of *AMPKα1* and *AMPKα2* alleles in e.g. gastrointestinal tissues (our unpublished data and) [89] and the haploinsufficiency of *LKB1* in polyp initiation (see Section 2) indicate it will be interesting to investigate whether $AMPK\alpha1^{-/-}$ and $AMPK\alpha2^{-/-}$ mice or tissue-specific double knockouts are susceptible to tumorigenesis.

5. LKB1 in regulating TGFβ-dependent cell differentiation

Peutz-Jeghers polyps contain a characteristic prominent smooth muscle core. The observation that polyp epithelial cells do not commonly carry secondary *LKB1* mutations (see above) indicated that it was not clear which cell types were important for polyp formation in carriers of germline *LKB1* mutations. Interestingly, smooth muscle specific deletion of *Lkb1* induces polyps with a prominent smooth muscle core and hyperproliferative epithelia [4] indicating the smooth muscle Lkb1 has a non-cell-autonomous role in controlling epithelial proliferation. *Lkb1* loss in stromal cells was associated with decreased TGFβ signaling and TGFβ production (Fig. 1) and lead to differentiation defects of the SMC-lineage [4,30]. Decreased TGFβ signaling to epithelial cells [4] may represent the mechanism allowing epithelial hyperproliferation and differentiation defects [5]. The polyps driven by stromal *Lkb1* deletion were indistinguishable from polyps in $Lkb1^{+/-}$ mice and PJS patients including the TGFβ signaling defects providing evidence that smooth muscle *LKB1* mutations are also critical in PJS polyposis. This is also supported by apparent lack of similar tumors upon epithelial *Lkb1* deletion [46,90]. Thus *Lkb1* can be considered a landscaper tumor suppressor [91] for PJS polyposis. Landscaper tumor suppression has been previously identified in mice with deletion of *TGFβRII* in fibroblasts leading to prostate and stomach carcinoma [92], and in mice with deletion of *Smad4* in T cells leading to epithelial tumors [93] with similarities with Juvenile Polyposis (JP) syndrome harboring *SMAD4* mutations.

In myofibroblasts LKB1 regulates TGFβ signaling between receptor activation and target gene activation [30]. Interestingly, the LKB1 substrate kinase NUAK2 has been reported to interact with TGFβRI, Smad2 and Smad4 suggesting that NUAK2 regulates recruitment or full activation of Smad's (Fig. 1) [94,95]. Alternatively, LKB1 substrate kinase phosphorylation and activation of p53 could be needed for proper activation of Smad dependent transcription [96].

The mode of LKB1 mediated regulation on TGFβ signaling is possibly cell/tissue specific as, similarly to stromal cells, LKB1 is critical for TGFβ signaling in endothelial cells *in vivo* [97], whereas attenuated LKB1 signaling was associated with enhanced TGFβ signaling in multiple epithelial cell lines [31,98]. Interestingly, downregulation of LKB1 in epithelial cell lines has been implicated in induction of epithelial to mesenchymal transition (EMT) [31,98,99], which could explain the increased metastasis of *Lkb1* mutated lung adenocarcinomas [13,31]. Possible mechanisms of TGFβ signaling suppression by LKB1 include the downregulation TGFβRI ALK5 by LKB1 substrate SIK1 [100] and inhibition of Smad4 when in a complex containing LKB1, LIP1 and Smad4 [41,98]. Whether the increased TGFβ signaling noted in some experimental systems is at play in gastrointestinal epithelial and whether it contributes to epithelial differentiation defects observed in *Lkb1* mutated mice [5,46] and PJS patients [5] requires further investigation.

6. LKB1 regulates cell polarity by controlling microtubule and actin dynamics

LKB1 and its orthologues have critical roles in regulation of cell polarity in several model systems. PAR-4, a *C. elegans* orthologue of LKB1, was identified as one of the six partitioning defective genes regulating asynchronous and asymmetric cell division of an early embryo [101], whereas drosophila LKB1 has been shown to regu-

late both oocyte and epithelial polarity [53]. Recent studies have indicated conservation of polarity regulation by LKB1 in mammalian cells (see below). Many changes resulting from defects in polarity including mitotic spindle defects, defective tissue integrity, epithelial to mesenchymal transition and subsequently increased cell motility are associated with cancer initiation and progression [102], and therefore it is important to consider these as a potential mechanism of LKB1 mediated tumor suppression.

Loss of asymmetric cell divisions are in part caused by deficient microtubule dynamics and deficient orientation of mitotic spindle. In *C. elegans* PAR-4 controls spindle pole positioning via activation of MARK kinase orthologue PAR-1 [101,103]. MARK kinases increase microtubule dynamics via phosphorylation of microtubule associated proteins (Fig. 1) [104] and MARK4 associates with microtubules and centrosomes [105]. In *Drosophila melanogaster* LKB1 is critical for mitotic spindle formation and morphology in larval brains [106] and S2 cells [107]. These effects could be mediated by SIK3 homologue CG15072 whose silencing mimics the LKB1 phenotype in S2 cells [107]. In mammalian cells LKB1 substrate kinases BRSK1 and SIK2 have been reported to localize to centrosomes where BRSK1 mediated phosphorylation on Ser-131 of gamma-tubulin [108] and SIK2 mediated phosphorylation on Ser-2392 of centrosomal linker protein C-Nap1 [109] are required for centrosome duplication and centrosome separation during mitosis, respectively (Fig. 1).

Thus LKB1 is implicated in regulation of several aspects of mitotic spindle formation and orientation. Deregulation of these processes often takes place in human cancer resulting in polyploidy and misorientation of the plane of division of epithelial cells. Studies on lung adenocarcinoma samples should reveal whether LKB1 mutated tumors demonstrate more severe mitotic spindle defects than LKB1 wild type tumors or whether mutations of LKB1 and other driver genes indicated in regulation of mitotic spindle are mutually exclusive.

Normal tight and adherence junctions promote epithelial polarity and prevent epithelial mesenchymal transition, which is associated with cancer progression. In MDCK cells downregulation of LKB1 and subsequent decrease in AMPK activation has been implicated in deficient formation of tight junctions upon calcium switch [110,111] and in bile canalicular network model LKB1–AMPK signaling activity is critical for polarity formation and maintenance [112]. LKB1 has been suggested to regulate also adherence junctions as *LKB1* nullizygosity leads to disorganized adherence junctions in *Drosophila* eye rhabdomeres [113] and LKB1 associates with adherence junctions in MDCK cells [54]. Furthermore, *LKB1* deletion in developing mouse pancreas with *Pdx1-Cre* leads to disruption of adherence and tight junctions and is correlated with cyst formation by acinar cells [45]. Here Lkb1 might be essential for cell–cell junction formation during development of the pancreas as defects in these have not been reported upon LKB1 deletion in the mature pancreas where, instead, deficient positioning of the nucleus and primary cilia were observed in response to *Lkb1* or *Mark2* deletion [114,115]. Cell–cell junction formation is essential for polarization and lumen formation of mammary epithelial structure. Indeed, polarization and lumen formation by MCF10A breast cancer cells is attenuated in a 3D model upon LKB1 knockdown [116] and, accordingly, *Lkb1* deletion in mouse mammary glands lead to development of ductal carcinomas [117]. Although *LKB1* mutations in sporadic breast cancers are rare [118], carriers of germline *LKB1* mutations have increased breast cancer incidence [7], which suggest a potential role for regulation of epithelial integrity in LKB1 tumor suppression.

Establishment and maintenance of cell–cell junctions are dependent on actin cytoskeleton and thus cell junction defects upon deregulation of LKB1 might reflect problems in actin dynamics. Here LKB1 is suggested to be required for myosin regulatory light chain (MLC/MRLC) phosphorylation on Thr18–Ser19 [119,120]. Phosphorylated MLC increases actomyosin contraction and actin stress fiber formation via activation of myosin, which triggers positive feedback loop between contraction and MLC phosphorylation [121]. Overexpression of a phospho-mimetic of Thr18 and Ser19 MLC rescues polarity and polyploidy defects of epithelial cells in LKB1 and AMPK mutant flies [122] and mimics LKB1 kinase complex dependent polarization of a single colorectal cancer cells [122,123]. Whether MLC is the direct target of AMPK remains still controversial [122,124]. On the other hand, NUAK1 and NUAK2 have been suggested to positively regulate MLC phosphorylation via inhibition of MLC-phosphatase complex. NUAK1 interacts with PP1 catalytic subunit and phosphorylates three sites on the regulatory subunit MYPT1 (PP1RC12A) to create 14-3-3 binding epitopes leading to sequestration of the phosphatase complex [125], whereas PP1 inhibition by NUAK2 is suggested to take place on actin stress fibers in MRIP dependent and partially kinase independent manner [120]. Here binding of phosphatase complex and NUAK2 on MRIP leads to inhibition of the phosphatase, which together with upregulation of NUAK2 levels in conditions of prominent stress fibers depicts a positive feedback loop resulting in increased stress fibers (Fig. 1) [120]. Accordingly, deletion of *Lkb1* in primary MEFs has been indicated in loss of actin stress fibers [30]. Thus it is plausible that NUAK1, NUAK2 and AMPK mediate LKB1 function in actin cytoskeleton regulation in a context dependent manner.

Deficient cell–cell junctions and altered actin and microtubule dynamics are associated with increased cell motility and cancer cell metastasis [126]. Indeed, *LKB1* mutations are correlated with aggressive phenotype of tumors [14,16] and cause increased invasiveness in mouse cancer models [13,23]. Both, microtubule destabilization by BRSK1-2 or MARK1-4 mediated phosphorylation on microtubule associated proteins [44,104] and reported increase in microtubule stability and polymerization upon AMPK mediated phosphorylation on microtubule + end motor CLP-170 [127] have been associated in regulation of cell migration. In a separate study overexpression of LKB1 mutants found in tumors interfered with microtubule polarization during astrocyte migration [128]. Also increased phosphorylation of MLC, which is controlled in part by NUAK kinases [120,125], and subsequently enhanced cell contractility has been shown to be critical determinant of cell migration. Actin and focal adhesions dynamics are reciprocally regulated and interestingly focal adhesion kinase activation has been associated with LKB1 inactivation in cell culture and *in vivo* [31]. The potential role of multiple LKB1 substrate kinases in regulation of cell motility calls for careful analysis of LKB1 substrate activation in space and time in motile cells to distinguish their roles.

7. Concluding remarks

Identification of frequent *LKB1* mutations in adenocarcinomas of the lung [20] and cervical cancer [16] has promoted *LKB1* as one of the central tumor suppressors. Because of apparent lack of LOH of *LKB1* loci at least in some tumor types [4,13,28], *LKB1* mutations might have been missed in earlier studies as LOH has been considered as criteria of tumor suppressor. Thus cancer genome and epigenetic screening approaches might reveal new tumor types carrying *LKB1* mutations. Accordingly, tumor sample screening for decreased LKB1 levels by immunohistochemistry has been used for identification of LKB1 deficient cancer types [10]. However, the lack of a well-established and controlled reagent for immunohistological staining of LKB1 has hampered wide-spread use of this useful approach. Thus efforts in production of better antibodies and perhaps even LKB1 activity assays will benefit studies on LKB1 deficiencies in variety of tumors and metastasis and will clarify in which cell types LKB1 levels are decreased in tumors.

It is not clear which LKB1 signaling pathways are involved in tumor suppression. Combining existing tumor models with overexpression of activated mutant or deletion of LKB1 substrate kinases could reveal the relevant LKB1 downstream targets inhibiting initiation and metastasis of tumorigenic cells *in vivo*. Also studies on *Lkb1* phosphorylation site mutant knock-in mice are likely to clarify the role of different upstream regulators in LKB1 tumor suppression. Studies on LKB1 will benefit cancer research but will also shed light on basic principles of metabolism and tissue integrity.

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