Targeting LKB1 signaling in cancer

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Abstract

The serine/threonine kinase LKB1 is a master kinase involved in cellular responses such as energy metabolism, cell polarity and cell growth. LKB1 regulates these crucial cellular responses mainly via AMPK/mTOR signaling. Germ-line mutations in LKB1 are associated with the predisposition of the Peutz–Jeghers syndrome in which patients develop gastrointestinal hamartomas and have an enormously increased risk for developing gastrointestinal, breast and gynecological cancers. In addition, somatic inactivation of LKB1 has been associated with sporadic cancers such as lung cancer. The exact mechanisms of LKB1-mediated tumor suppression remain so far unidentified; however, the inability to activate AMPK and the resulting mTOR hyperactivation has been detected in PJS-associated lesions. Therefore, targeting LKB1 in cancer is now mainly focusing on the activation of AMPK and inactivation of mTOR. Preclinical in vitro and in vivo studies show encouraging results regarding these approaches, which have even progressed to the initiation of a few clinical trials. In this review, we describe the functions, regulation and downstream signaling of LKB1, and its role in hereditary and sporadic cancers. In addition, we provide an overview of several AMPK activators, mTOR inhibitors and additional mechanisms to target LKB1 signaling, and describe the effect of these compounds on cancer cells. Overall, we will explain the current strategies attempting to find a way of treating LKB1-associated cancer.

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1. Introduction

1.1. The Peutz–Jeghers syndrome

In 1921, the Dutch physician Johannes Peutz was the first to describe the combination of intestinal polyps, pigmented mucocutaneous lesions and heredity [1]. In 1949, Harold Jeghers specified the description of the syndrome [2], and in 1954 the eponym “Peutz–Jeghers syndrome” (PJS) was first used. PJS is a rare autosomal dominant disorder with a prevalence of 1 in 8300–280,000 [3]. The PJS-associated mucocutaneous hyperpigmentation is predominantly apparent on the lips, oral mucosa, face and/or hands at young ages, and can diminish or disappear in adulthood. This pigmentation has unknown clinical implications but it can be used in the diagnosis of PJS.

1.2. Gastrointestinal hamartomas in PJS

All PJS patients develop early-onset hamartomatous polyposis throughout the entire gastrointestinal (GI) tract. Peutz–Jeghers polyps, or hamartomas, have a relatively benign appearance, but with a markedly overgrowth of core smooth muscle bundles (Fig. 1a). Hamartomatous polyps are clearly distinct from the more common adenomatous polyps, which are premalignant lesions characterized by a dysplastic epithelium (‘adenoma-to-carcinoma sequence’). In contrast, the overlying epithelium in hamartomatous polyps is usually well differentiated but it can be hyperplastic [4]. Adenomatous and carcinomatous changes in PJS-hamartomas have been reported, though rarely [5–7]. Therefore, the gastrointestinal hamartomas and carcinomas developed in PJS patients are believed to be two distinct entities, though this is still debated. PJS-hamartomas are most prevalent (64–90%) in the small intestine (jejunum > ileum > duodenum), but up to 50% of the patients also develop hamartomas in the stomach and colon. Also, hamartomatous polyps have been detected in the nasal mucosa, lungs and bladder [8]. GI hamartomas may develop in young children, but typically around the time of early adolescence. Despite the fact that these hamartomas follow a relatively benign course, they can cause severe complications, such as abdominal pain, rectal bleeding, anemia, small intestinal intussusception, bowel obstruction, and rectal prolapse of these polyps. Due to the possible bowel obstructions, PJS patients require routine endoscopic surveillance of the GI tract to remove significant polyps [8,9].

1.3. Cancer risk in PJS patients

Patients predisposed for PJS are also at increased risk for developing cancer at a relatively young age, and at an age of 70 years a cumulative risk of 37–93% has been determined for these patients [10,11] (Fig. 1b). A wide spectrum of malignancies has been described, with the highest cumulative risks attributed to cancers of gastrointestinal (38–93%) (Fig. 1b), breast (32–54%) or gynecological (13–18%) origin [10]. The most frequently observed gastrointestinal cancers constitute colorectal cancer (CRC), but also small intestinal, gastric and pancreatic are observed more frequently compared with the general population [10–12]. In addition, rare tumors such as testicular or ovarian sex cord tumors, Sertoli-cell tumors and adenoma malignum of the uterine cervix have been associated with PJS.

1.4. The tumor suppressor gene LKB1

In 1997, the PJS susceptibility locus was mapped to chromosome 19p13 [13]. One year later, inactivating germ-line mutations in the serine/threonine kinase 11 (STK11) gene – from hereon referred to as liver kinase B1 (LKB1) gene – were detected to cause predisposition for PJS [14,15]. With the currently available techniques, an LKB1 germ-line mutation can be detected in approximately 80–94% of clinically affected PJS families. Around 25% of cases are sporadic, thought to be due to de novo germ-line LKB1 mutations. Around 150 different mutations in LKB1 associated with PJS have been detected, without a clear genotype–phenotype correlation. The majority of mutations result in truncation or abnormal splicing, although in approximately 20% of the cases a missense mutation in the kinase domain of LKB1 is detected. It has been suggested that truncating mutations tend to associate with an earlier age of onset of disease as compared with PJS cases associated with missense mutations in LKB1 [16]. For a subset of PJS-related mutations it has been shown that they impair the
capacity of autophosphorylation and function of the LKB1 protein, which is described elsewhere in this review.

The original studies characterizing Lkb1 function in mice showed that Lkb1+/− mice die at embryonic stage, highlighting a crucial role during early development [17]. Although loss of Lkb1 prevented Ras/Large-T-induced transformation in mouse embryonic fibroblasts (MEFs) [18], it enhanced PJS-like polyp formation in the GI tract in Lkb1−/− mice [18,19]. The interesting conclusion from those pioneering studies is that LKB1 is not likely to be a driver mutation in cancer, but as a secondary mutation it might enhance tumorigenesis.

LKB1 is classified as a tumor suppressor gene, implying that both alleles need to be inactivated to contribute to tumor development. Loss of heterozygosity (LOH) of the remaining LKB1 allele has been detected in PJS-hamartomas, but it is observed more frequently in carcinomas [20,21]. This suggests that bi-allelic loss of LKB1 is not necessary for hamartomatous polyp development, but favors progression to carcinoma. Therefore, LKB1 is suggested to act as a haplo-insufficient tumor suppressor gene. Because LOH does not necessarily take place in the invading epithelial cancer cells, there is still some controversy about where LKB1 loss contributes to carcinogenesis in PJS patients.

The haplo-insufficiency of Lkb1 has been confirmed in mice, where neither loss of the wild-type Lkb1 allele nor loss of expression of Lkb1 could be detected in most gastrointestinal polyps of heterozygous Lkb1 knockout mice [18,19,22]. These results suggest that partial loss of Lkb1 is sufficient for tumor development. Haplo-insufficient tumor suppressor genes usually evoke their role in tumorigenesis in the context of additional oncogenic triggers. In the case of Lkb1, it has been shown that additional loss of Pten or p53, or additional oncogenic activation of Kras synergizes with Lkb1 loss in order to promote tumor formation [23–26].

In a subset of gastrointestinal hamartomas of heterozygous Lkb1 knockout mice loss of the wildtype Lkb1 allele or Lkb1 expression could be detected specifically in the epithelial compartment, suggesting that Lkb1 exerts its tumor suppressive functions mainly in the epithelium [18]. However, myofibroblast-specific loss of Lkb1 in mice has been shown to be sufficient for gastrointestinal hamartoma development [27], indicating that Lkb1 suppresses tumor formation through signaling in mesenchymal cells as well. No phenotypical differences were observed between polyps of mice with mono-allelic or bi-allelic Lkb1 deletion, suggesting that also in stromal cells Lkb1 acts as a haplo-insufficient tumor suppressor [27]. Lkb1-deficient mesenchymal cells stop producing TGFβ, which is a crucial factor suppressing tumor initiation and progression. Interestingly, Lkb1 has recently been shown to inhibit Smad4-mediated transcriptional activation of TGFβ targets in epithelial cells [28], which might indicate that LKB1 controls TGFβ signaling at both ends. However no strategies for the moment have been devised for targeting the TGFβ pathway, likely due to its more complex role in cancer late progression.

2. LKB1 in sporadic cancer

Despite the strong association between LKB1 mutations and increased risk for carcinogenesis in PJS, LKB1 is not commonly mutated somatically in sporadic cancers, except for lung cancer (Table 1). In contrast, allelic loss, LKB1 promoter hypermethylation or reduced LKB1 expression is observed in a wide variety of sporadic cancers (reviewed in [29,30]) (Table 1). Because of the suggested haplo-insufficiency of LKB1, this could indicate that loss of LKB1, probably in combination with additional oncogenic events, is involved in the development and/or progression of these sporadic cancer types.

2.1. Colorectal cancer

Since PJS patients have an increased risk for developing CRC [11], LKB1 mutation analyses have been set-up for sporadic CRC as well. However, somatic LKB1 mutations have been detected sparsely in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Frequencies of LKB1 mutations/deletions and of LOH of 19p13.3 reported in sporadic carcinomas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of sporadic cancer</td>
<td>Mutation/deletion</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td>0/20 (0%)</td>
</tr>
<tr>
<td></td>
<td>0/72 (0%)</td>
</tr>
<tr>
<td></td>
<td>7/13 (54%)</td>
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<td>1/71 (1%)</td>
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<td>1/80 (1%)</td>
</tr>
<tr>
<td></td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Small intestinal</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td></td>
<td>5/23 (22%)</td>
</tr>
<tr>
<td>Gastric</td>
<td>3/28 (11%)</td>
</tr>
<tr>
<td></td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>0/40 (0%)</td>
</tr>
<tr>
<td></td>
<td>3/103 (3%)</td>
</tr>
<tr>
<td></td>
<td>1/20 (5%)b</td>
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<tr>
<td></td>
<td>0/5 (0%)c</td>
</tr>
<tr>
<td>Breast and gynecological</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>0/62 (0%)</td>
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<tr>
<td></td>
<td>5/30 (17%)</td>
</tr>
<tr>
<td></td>
<td>9/16 (56%)a</td>
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<tr>
<td>Ovarian</td>
<td>0/45 (0%)</td>
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<tr>
<td>SCTAT</td>
<td>0/12 (0%)d</td>
</tr>
<tr>
<td></td>
<td>1/12 (8%)e</td>
</tr>
<tr>
<td></td>
<td>0/5 (0%)f</td>
</tr>
<tr>
<td></td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Cervical</td>
<td>1/26 (4%)</td>
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<tr>
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<td>0/8 (0%)a</td>
</tr>
<tr>
<td>Testicular</td>
<td>1/28 (4%)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>NSCLC—adenocarcinoma</td>
<td>1/12 (8%)</td>
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<tr>
<td></td>
<td>5/20 (25%)</td>
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<td></td>
<td>7/155 (5%)</td>
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<td></td>
<td>27/80 (34%)</td>
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<td></td>
<td>3/81 (4%)</td>
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<td></td>
<td>13/207 (6%)</td>
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<td></td>
<td>0/51 (0%)</td>
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<td></td>
<td>4/7 (57%)f</td>
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<td>NSCLC—squamous cell</td>
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<td>8/42 (19%)</td>
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<td>5/92 (5%)</td>
</tr>
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<td></td>
<td>6/67 (9%)</td>
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<tr>
<td>NSCLC—large cell</td>
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<tr>
<td></td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td></td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td></td>
<td>3/11 (27%)</td>
</tr>
<tr>
<td>SCLC</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td></td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Additional</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td></td>
<td>1/15 (7%)</td>
</tr>
<tr>
<td></td>
<td>2/35 (6%)</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>0/24 (0%)</td>
</tr>
<tr>
<td>Renal</td>
<td>0/19 (0%)</td>
</tr>
<tr>
<td>Brain</td>
<td>31/248 (13%)a</td>
</tr>
</tbody>
</table>

CRC, colorectal carcinoma; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; SCTAT, sex-cord stromal tumors of the ovary.
a Brain metastases from this cancer type.
b Intraductal papillary mucinous neoplasms.
c Pancreatic acinar cell carcinomas.
d Ovarian granulosa cell tumors.
e Adenoma malignum of uterine cervix.
f Mucinous bronchioloalveolar carcinomas.

Various histological subtypes of which LOH was most frequently observed in gliomas (17%) and pituitary adenomas (19%).
these carcinomas (Table 1). In contrast, one study reported a mutation frequency of 54% in carcinomas in the left-sided colon [31]. Therefore it was suggested that LKB1 plays an important role in left-sided colon cancer carcinogenesis; however, in a later study no somatic LKB1 mutations could be detected in 50 left-sided colon carcinomas [32] (Table 1). LOH of 19p13.3 has been detected more frequently in sporadic CRC with frequencies ranging from 13% to 53% (Table 1). This suggests that reduced levels of LKB1 may contribute to the development of sporadic CRC, though not through mutational inactivation. Lkb1-deficient mice did not develop intestinal carcinomas, nor did the hamartomatous polyps progress to a more malignant histological phenotype when p53 was additionally deleted [25,26]. Therefore, it remains elusive how loss of LKB1 contributes to colorectal carcinogenesis.

2.2. Pancreatic cancer

PJS patients have an increased risk for developing pancreatic cancer, but somatic LKB1 mutations have been detected only in a small proportion of sporadic pancreatic carcinomas (Table 1). In addition, a low frequency of reduced LKB1 expression has been observed in these carcinomas [33]. However, reintroduction of LKB1 expression in LKB1-silenced pancreatic cancer cells induced apoptosis, suggesting that LKB1 suppresses pancreatic cancer cell survival in vitro [34]. Deletion of Lkb1 in murine pancreatic epithelial cells of the islets, ducts, and acini resulted in the development of ductal metaplasia and cystadenomas, but no formation of pancreatic intraepithelial neoplasia (PanIN) or adenocarcinomas [24,35]. Introduction of an oncogenic Kras mutation in heterozygous Lkb1 knockout mice resulted in formation of pancreatic ductal adenocarcinomas, indicating that Lkb1 is a haplo-insufficient tumor suppressor and cooperates with Kras in the development of pancreatic cancer in mice [24].

2.3. Breast cancer

Somatic mutations in LKB1 and LOH of 19p13 are not observed frequently in primary sporadic breast carcinomas; however, LOH was frequently detected in brain metastases from breast carcinomas (Table 1). In addition, it has been shown that LOH of the LKB1 locus in primary breast carcinomas increased significantly as the tumors progressed to poorer histological grade, and that low LKB1 expression in breast carcinomas is associated with poorer histological grade, presence of lymph node metastasis and a shorter overall survival [36–38]. In line with these observations, it has been shown that LKB1 suppresses breast cancer cell migration and invasion in vitro, and tumor growth, microvessel density and lung metastasis in vivo [39,40]. Together, this suggests that loss of LKB1 is a late event in breast cancer and promotes breast cancer progression.

2.4. Lung cancer

Despite the fact that lung cancer is not clearly associated with PJS, LKB1 is the third most frequently mutated gene in sporadic non-small cell lung carcinomas (NSCLC). Though the proportion of missense mutations seems to be higher among the somatic mutations in sporadic cancer (45%) than among the germ-line mutations in PJS patients (21%), the mutations detected in NSCLC comprise mainly nonsense mutations or a combination of indels or large intragenic deletions on one LKB1 allele – resulting in truncation of the protein – plus large chromosomal deletions on the other allele [30,41]. Somatic LKB1 mutations are most frequently detected in adenocarcinomas, but also in other histological subtypes of NSCLC (Table 1). Notably, LKB1 mutations are more frequently observed in NSCLC of Caucasians compared with East Asian populations (reviewed in [42]). In addition, LKB1 mutations frequently coincide with KRAS mutations. NSCLC patients with LKB1; KRAS compound mutations tend to have a poorer prognosis compared with patients with KRAS-mutated NSCLC without a concomitant LKB1 mutation, suggesting that loss of LKB1 induces more aggressive tumor phenotypes [43,44]. This is also shown in mice where double mutant Lkb1; Kras mice develop more aggressive tumors with higher tumor multiplicity, multiple NSCLC histologies and more frequent metastasis [45].

Overall, loss of LKB1 is involved in cancers associated with PJS as well as with a variety of sporadic malignancies, where it is associated with tumor progression. Therefore, LKB1 and its downstream signaling pathways suit a perfect target for therapeutic intervention of these cancers.

3. LKB1 signaling and function

3.1. The LKB1 protein

LKB1 encodes a 48 kDa protein, LKB1, which is ubiquitously expressed in adult as well as fetal tissues, particularly in pancreas, liver, testes and skeletal muscles [14,15]. It contains an evolutionary conserved serine/threonine kinase domain (Fig. 2), in which the vast majority of PJS-associated missense mutations are located, resulting in impaired kinase activity and cell growth suppressive capacity [15,68–70]. In addition to the mutations detected in the kinase domain, a substantial proportion of PJS-associated mutation as well as mutations detected in sporadic cancers is located in the C-terminus of LKB1. These mutations have been shown not to impair kinase activity or its ability to promote growth arrest [71]. In this C-terminal region of LKB1, some posttranslational modification sites have been identified. First, four autophosphorylation sites have been described, i.e. threonine (Thr) 185, Thr189, Thr336 and Thr402 [72,73] (Fig. 2). Autophosphorylation of Thr336 seems not to affect its catalytic activity nor its cellular localization; however, it may inhibit the cell growth suppressive capacity of LKB1 [73]. In addition, four phosphorylation sites have been identified, i.e. serine (Ser) 31, Ser325, Thr363 (mouse Thr366) and Ser428 (mouse Ser431) [73–75] (Fig. 2). Thr363 is phosphorylated by ATM in response to ionizing radiation [76]. Phosphorylation of Ser428 by cAMP-dependent kinase (PKA), p90RSK, and protein kinase Czeta (PKCζ) is not affecting its catalytic activity, but it is essential for LKB1 to inhibit cell growth [74,75,77]. The kinases for Ser31 and Ser325 have so far not been identified. Finally, a prenylation site, i.e. Cys430 (mouse Cys433), has been identified [75] (Fig. 2). Since it has been shown that farnesylation at Cys430 is not essential for LKB1 to suppress cell growth, the functional relevance of this prenylation is not yet understood [74].
3.2. LKB1 activation

LKB1 contains a nuclear localization signal domain which is likely to be the reason that LKB1 is normally localized in the nucleus [78] (Figs. 2, 3). Because LKB1 lacks a nuclear export domain of its own, it needs interaction with other proteins in order to be actively exported out of the nucleus. Activation of LKB1 is therefore associated with its translocation to the cytoplasm which is induced upon formation of a heterotrimer with the STE20-related adaptor (STRADα) and scaffolding mouse 25 (MO25) proteins [68,72] (Fig. 3). By facilitating the binding of exportins to LKB1 and acting as a competitor for importin-α/β, STRADα prevents nuclear re-localization of LKB1. MO25 merely serves as a stabilizer of the LKB1–STRAD interaction [68]. When in complex with STRADα and MO25 and located in the cytoplasm, LKB1 phosphorylates and activates kinases of the AMP-activated kinase (AMPK) family, i.e. AMPKα1, AMPKα2, NUAK1, NUAK2, SIK1, SIK2, QSK, MARK1, MARK2, MARK3, MARK4, BRSK1, BRSK2 and SNRK [79–81] (Fig. 3). Notably, for several PJS-associated mutations, it has been shown that the resulting LKB1 mutants are retained in the nucleus [15,68–70].

3.3. LKB1 in cell polarity

Originally, the Caenorhabditis elegans and Drosophila melanogaster counterparts of LKB1, Par-4 and dlkβ1 respectively, have been shown to play major roles in cellular polarity [82,83]. LKB1 induces epithelial-cell polarization through sorting of apical and basolateral membrane proteins, formation of epithelial junctions and reorganization of the actin cytoskeleton, which is at least in part mediated via activation of AMPKα [84–89]. In addition to activation of AMPKα, LKB1 regulates polarization through the activation of MARK isoforms (mammalian counterparts of C. elegans and D. melanogaster Par-1), which play a remarkable role in microtubule skeleton organization [90,91]. Through activation of BRSk (or SAD) proteins, LKB1 regulates neuronal migration and axonal outgrowth [92,93]. A role for C. elegans and D. melanogaster Par-4/dlkβ1 in asymmetric positioning of the mitotic spindle and cytoplasmic determinants during mitosis has been established [94].

In mammalian cells, LKB1 has recently been shown to be involved in epithelial polarization by controlling Rho GTPases, primary cilium, phosphoinositide and Wnt/GSK3β signaling [95–98]. More specifically, LKB1 is involved in centrosome positioning, lumen initiation and brush border formation during epithelial morphogenesis [96,97]. Together this shows that LKB1 acts as a master kinase regulating neuronal and epithelial polarization, polarized cell migration and asymmetric cell division (Fig. 4). Given the role of LKB1 in these attributions, normal LKB1 function is required for proper function and proliferation of epithelial tissues. Defects in LKB1 may therefore result in tissue disorganization associated with cancer [99–101]. Notably, PJS-associated mutations as well as mutations detected in sporadic cancers located in the C-terminus of LKB1 have been shown to impair cellular polarity [71].

3.4. LKB1 in energy metabolism

In addition to the regulation of cellular polarity, AMPK signaling controls lipid and glucose metabolism (Fig. 4). In circumstances of energy stress due to either excessive ATP consumption, or reduced aerobic ATP production, e.g. in the case of hypoxia, cellular AMP/ATP ratios increase. This is sensed by AMPKγ which binds AMP, leading to the complex formation of AMPKα–β–γ subunits [102–104]. Threonine residue 172 in the activation loop of AMPKα is now accessible to be phosphorylated by LKB1 [105–107]. Activated AMPK controls metabolic processes such as isoprenoid, fatty acid and glyco-gogen synthesis via regulation of downstream targets such as HMG-CoA reductase, acetate CoA carboxylase (ACC) and glycerogen synthase [108–110]. Thus, by suppressing energy-consuming processes on the one hand, and enhancing energy gaining pathways on the other, AMPK activation by LKB1 aids in restoration of the cellular energy status.

3.5. LKB1 in cell growth

Moreover, LKB1 has been associated with cell growth control via multiple different signaling pathways (Fig. 4). One such downstream pathway of LKB1/AMPK is TSC/mTOR signaling [111]. Activated AMPK phosphorylates TSC2, thereby activating the TSC1:TSC2 complex [112], which in turn regulates the activity of the mTORC1, a complex consisting of mTOR, raptor and mLST8. The activated TSC1: TSC2 complex, which expresses GTPase activity towards Rheb, a small G-protein that promotes mTORC1 activity when GTP-bound, induces conversion of active GTP-bound Rheb to inactive GDP-bound Rheb which subsequently results in inhibition of mTORC1 [113]. In addition to inhibiting mTORC1 via phosphorylation of TSC2, AMPK directly phosphorylates raptor resulting in the inhibition of mTORC1 [114]. mTORC1 plays a key role in protein translation by phosphorylating and activating the ribosomal protein S6 kinase (S6K), and through
the inhibition of eukaryotic initiation factor 4E binding proteins (4E-BPs) [115]. In addition, mTORC1 activation stimulates angiogenesis by stabilizing hypoxia-inducible factor 1α (HIF-1α) in conditions of hypoxia [116]. Furthermore, mTORC1 activity inhibits autophagy via phosphorylation of ATG13 and ULK1/2 [117,118]. Thus, LKB1 regulates mTOR signaling to control cell growth via different pathways and cellular responses. However, recently, it has been shown that LKB1 is necessary to maintain hematopoietic stem cell (HSC) homeostasis mainly independent of AMPK and mTOR [119–121], indicating that also other molecular pathways are involved in LKB1-regulated cell growth (Fig. 4).

LKB1 is also involved in cell cycle regulation and apoptosis. Re-introduction of LKB1 expression in cells lacking LKB1 induces cell cycle arrest in G1 [37,122–127], while knocking down LKB1 expression triggers cell cycle progression from G1 to S phase [128]. Notably, re-introduction of LKB1 catalytic deficient mutants in CRC cells could not induce cell cycle arrest, but it even upregulated expression of the cell cycle inducer cyclin D [127]. LKB1-induced cell cycle arrest is mediated by upregulation of the cell cycle inhibitors p21 and/or p27 [37,122–125,127,129]. In addition, Lkb1-mediated tumor promotion has been shown to be mediated by suppression of p21-dependent growth arrest in vivo [24]. LKB1-mediated cell cycle regulation has been shown to be regulated via p53-dependent and p53-independent mechanisms [122,123,126,127,129,130]. Additionally, LKB1 has been shown to interact with and phosphorylate p53, and mediate p53-induced apoptosis [132,131,132], while in Drosophila dLkb1 induces p53-independent apoptosis via activation of the JNK pathway [133]. In mice, compound loss of p53 and Lkb1 accelerates onset and increased incidence of polyplis, indicating that Lkb1 and p53 cooperate in tumor development [25,26]. PJS-associated LKB1 mutants have been shown to diminish p53 activity [134]. Thus, LKB1 suppresses cell cycle progression and induces apoptosis most likely via suppression of p21, though the role of p53 in these processes remains contradictory (Fig. 4).

PJS and Cowden syndrome are both hereditary hamartomatous polyposis syndromes. In Cowden syndrome, patients are affected by a germ-line mutation in phosphatase and tensin homologue (PTEN), a phosphatase involved in the PI3K/AKT survival pathway. Although these syndromes overlap in their clinical characteristics, they also have distinct features [135]. Several studies in cells and in mice have shown that LKB1 acts upstream of PTEN (Fig. 4). In fact, LKB1 has been shown to interact with and phosphorylate PTEN, which was disrupted by introducing PJS-associated mutations in LKB1 [136,137]. In addition, LKB1 induces expression and nuclear export of PTEN resulting in reduced PI3K/AKT signaling and apoptosis [124,137,138]. This LKB1-mediated nuclear export of PTEN has been shown to be independent of AMPK/mTOR signaling [138]. In vivo, loss of Lkb1 cooperates with Pten loss to accelerate tumorigenesis [23,139]. The tumorigenesis in these compound mutant mice was shown to be, at least in part, mediated by mTOR signaling since treatment with mTOR inhibitors reduced tumor formation [139,140].

These studies reveal a pleiotropic role for LKB1 in cell polarity, energy metabolism, and cell growth, processes that are deregulated in cancer. Most of these processes are mediated via AMPK/mTOR signaling, suggesting that this major downstream pathway is a suitable candidate to target for therapy against LKB1-associated cancer. However, other responses induced by loss of LKB1 have been shown to be independent of AMPK and/or mTOR signaling, suggesting that the tumor suppression functions of LKB1 are, at least in part, also mediated via other downstream effectors.

### 4. Targeting LKB1 signaling in cancer

Since various cancers have been associated with impaired AMPK activation and/or mTOR inhibition, whether or not triggered by loss of LKB1, AMPK/mTOR signaling has been suggested to serve a suitable target for cancer treatment. Both pharmacological AMPK activators (metformin) and mTOR inhibitors (rapamycin and its analogs sirolimus, everolimus and temsirolimus) are available and used in clinical settings. In addition to these clinically approved drugs, additional compounds affecting LKB1 signaling have been identified and are being tested in preclinical settings for their efficacy as anti-cancer agents. Since the discovery of LKB1 as the causative gene for PJS, and its signaling routes regulating cell growth, several studies have been focusing on targeting LKB1/AMPK/mTOR signaling in order to treat PJS-associated tumors.

#### 4.1. Metformin

In the 1970s, metformin, a biguanide (Fig. 5a), was approved by the Food and Drugs Administration (FDA) for the treatment of Diabetes Mellitus type 2 (DM2) in Europe, and in 1995 in the USA. Since then, millions of persons are using metformin, which has been shown to increase overall survival and prevent macrovascular complications in DM2 patients [141]. Metformin is also used successfully in polycystic ovarian syndrome (PCOS) and the management of the metabolic syndrome [142,143]. The efficacy of metformin in these metabolic disorders is attributed to the potential of metformin to reduce hepatic glucoseogenesis and improve insulin sensitivity [144]. Metformin has been shown to be an activator of AMPK, which is one of the master regulators of these metabolic processes (Fig. 5c) [145]. The interest in metformin as an anti-cancer drug arose when population studies showed that metformin use is associated with a significant reduction of neoplasms in general, and of breast, pancreatic and prostate cancers in particular [144,146,147]. Therefore, metformin might also serve as an anti-tumor agent in cancer prevention and treatment.

The anti-tumor effect of metformin could partly be explained by its action in improving blood glucose and insulin levels [148]. However, metformin induces more direct anti-tumor responses in cancer cells as well. Several in vitro studies have shown that metformin treatment inhibits cell growth, induces apoptosis, and reduces migration and invasion in a variety of human cancer cell lines (Table 2). The anti-proliferative effects have been shown to be mediated via inhibition of insulin-like growth factor-1 (IGF-1) signaling [149–152], but also via suppression of genes inducing cell cycle S- and M- phases [153–155]. The apoptosis-inducing effects of metformin have been
reported to be p53-dependent, mediated by downregulation of the Bcl-2 protein family, targeting ERK and STAT3 signaling, and through activation of the JNK/p38MAPK pathway [156–160].

In addition to these anti-proliferative and pro-apoptotic effects of metformin in vitro, metformin treatment reduces growth of various tumor types and/or delays tumor onset in animal models (Table 3). Conductory effects on angiogenesis and metastasis have been reported in xenograft mouse models (Table 3). Metformin treatment reduced tumor growth, microvessel density and metastasis in ovarian cell tumors, while it promoted tumor growth and the angiogenic phenotype in Erα-negative breast cancer cell tumors [161,162] (Table 3). In another high-energy diet fed xenograft mouse model, metformin did reduce growth of primary triple-negative breast cancer cell tumors, but did not affect metastasis [163] (Table 3). These results suggest that the effects of metformin on tumor progression depend on the tumor type and/or additional circumstances such as hormonal and metabolic status. Notably, metformin potentiates the effects of chemotherapeutic agents, such as cisplatin, paclitaxel, doxorubicin and gemcitabine, suggesting that metformin serves a potential adjuvant in conventional chemotherapy [161,164–169].

Most of the in vitro and in vivo anti-tumoral actions of metformin have been associated with AMPK activation and/or reduction in mTOR activity [158,170–173]. However, other studies specifically report AMPK-independent mechanisms of action of metformin as well [174–176]. Even though AMPK is activated upon metformin treatment, AMPK is not the direct target of metformin. Metformin is suggested to directly target complex I of the mitochondrial respiratory chain which evokes a rise in cellular AMP:ATP ratios [177,178]. This subsequently potentiates AMPK to be phosphorylated and activated by LKB1. Interestingly, metformin has been shown to induce LKB1 cytosolic translocation [134,179]. Contradictory results are reported about the requirement of LKB1 in metformin-induced AMPK activation and subsequent effects on metabolism in cells [23,175,179–183]. However, in women with PCOS single nucleotide polymorphisms in LKB1 were associated with a significantly reduced response to metformin treatment [184]. Moreover, metformin failed to inhibit cell growth in cells lacking LKB1, indicating that LKB1 is required for metformin-induced growth inhibitory effects [23,175]. Therefore, it is proposed that metformin is not a suitable drug for the treatment of tumors showing bi-allelic loss of LKB1 and/or loss of LKB1 expression, as is often the case in PJS patients. In these patients, metformin might, however, be a useful drug to prevent hamartoma and carcinomatous development and outgrowth, although studies testing this in Lkb1-mouse models and in PJS patients have not yet been performed. Although scarce, clinical evidence for anti-cancer effects of metformin have been reported for sporadic cancer. Metformin use suppressed colonic epithelial proliferation and formation of rectal aberrant crypt foci, an early feature of CRC, in non-diabetic patients [170]. More recently, anti-proliferative effects of metformin use in non-diabetic women with operable invasive breast cancer have been described [185]. All epidemiologic, preclinical and clinical evidence has led to the design of a number of prospective clinical trials investigating metformin therapy for cancer in the neoadjuvant and adjuvant (in combination with standard chemotherapy or hormone therapy) settings (www.clinicaltrials.gov). The majority of studies concern breast, prostate, and pancreatic cancer, in line with previous observations of population studies. Two phase II trials investigate the use of metformin as chemopreventive agent in pre-malignant conditions, i.e. Barrett esophagus (NCT01447927) and colorectal adenomas (NCT01312467). In addition, one phase III trial is currently recruiting participants (NCT01101438), evaluating the effects of metformin for early-stage breast cancer outcomes, including recurrence and death. Results of these trials are awaited.

In addition to metformin, other compounds such as TZDs [186], statins [187] and D942 [188] have been shown to activate AMPK indirectly by

<table>
<thead>
<tr>
<th>Cancer cell type</th>
<th>Inhibiting cell growth</th>
<th>Inducing apoptosis</th>
<th>Inhibiting migration/invasion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast&lt;sup&gt;a&lt;/sup&gt;</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>[150,154,155,157,160,203–206]</td>
</tr>
<tr>
<td>Pancreas</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>[151,152,207,208]</td>
</tr>
<tr>
<td>Colon&lt;sup&gt;b&lt;/sup&gt;</td>
<td>√</td>
<td>√</td>
<td></td>
<td>[171,209]</td>
</tr>
<tr>
<td>Stomach</td>
<td>√</td>
<td></td>
<td></td>
<td>[210]</td>
</tr>
<tr>
<td>Lung&lt;sup&gt;c&lt;/sup&gt;</td>
<td>√</td>
<td></td>
<td></td>
<td>[149,156]</td>
</tr>
<tr>
<td>Ovary</td>
<td>√</td>
<td></td>
<td></td>
<td>[159]</td>
</tr>
<tr>
<td>Prostate</td>
<td>√</td>
<td></td>
<td></td>
<td>[158,171]</td>
</tr>
<tr>
<td>Endometrium</td>
<td>√</td>
<td></td>
<td></td>
<td>[211]</td>
</tr>
<tr>
<td>Leukemia&lt;sup&gt;d&lt;/sup&gt;</td>
<td>√</td>
<td></td>
<td></td>
<td>[212]</td>
</tr>
</tbody>
</table>

<sup>a</sup> Part of the actions have been detected in triple-negative breast cancer cells.

<sup>b</sup> These actions have been detected in P53-deficient colon cancer cells specifically.

<sup>c</sup> Non-small cell lung cancer cells.

<sup>d</sup> BCR-ABL-positive chronic myeloid leukemia cells.

---

Table 2

<table>
<thead>
<tr>
<th>Metformin treatment-induced cellular responses in cancer cell lines.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer cell type</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Breast&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Colon&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Lung&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ovary</td>
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<tr>
<td>Prostate</td>
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<tr>
<td>Endometrium</td>
</tr>
<tr>
<td>Leukemia&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Part of the actions have been detected in triple-negative breast cancer cells.

<sup>b</sup> These actions have been detected in P53-deficient colon cancer cells specifically.

<sup>c</sup> Non-small cell lung cancer cells.

<sup>d</sup> BCR-ABL-positive chronic myeloid leukemia cells.

---

Table 3

Metformin treatment in animal models.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Model</th>
<th>Effect of metformin treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>Nu/nue mice xenograft MiaPaca-2 cells, Panc-1 cells</td>
<td>Reduced tumor growth</td>
<td>[152]</td>
</tr>
<tr>
<td></td>
<td>Nu/nue mice xenograft LNCaP cells</td>
<td>Reduced tumor growth</td>
<td>[213]</td>
</tr>
<tr>
<td></td>
<td>Syrian golden hamsters + BOP + high-fat diet</td>
<td>Reduced tumor incidence</td>
<td>[214]</td>
</tr>
<tr>
<td>Colon/intestinal</td>
<td>Nu/nue mice xenograft HCT116 cells</td>
<td>Reduced tumor growth</td>
<td>[209]</td>
</tr>
<tr>
<td></td>
<td>C37/BL6 mice xenograft MC38 cells + high-energy diet</td>
<td>Reduced tumor growth</td>
<td>[215]</td>
</tr>
<tr>
<td>Breast</td>
<td>Nu/nue mice xenograft MDA-MB-231 cellsl&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Reduced tumor growth</td>
<td>[203]</td>
</tr>
<tr>
<td></td>
<td>Nu/nue mice xenograft MDA-MB-435 cellsc&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Increased tumor growth, increased angiogenesis</td>
<td>[162]</td>
</tr>
<tr>
<td></td>
<td>Balb/c mice + AOM</td>
<td>Decreased incidence of large (&gt;2 mm) adenomas</td>
<td>[217]</td>
</tr>
<tr>
<td></td>
<td>Apc&lt;sub&gt;Min&lt;/sub&gt;−/− mice</td>
<td>Reduced tumor growth</td>
<td>[217]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Nu/nue mice xenograft A2780 cells</td>
<td>Reduced tumor growth, and microvessel density, and metastatic nodules</td>
<td>[161]</td>
</tr>
<tr>
<td>Gastric</td>
<td>Nu/nue mice xenograft MKN74 cells</td>
<td>Reduced tumor growth</td>
<td>[210]</td>
</tr>
<tr>
<td>Lung</td>
<td>A/J mice + NNK</td>
<td>Reduced tumor growth</td>
<td>[220]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>C37/BL6 mice xenograft B16 cells</td>
<td>Reduced tumor growth</td>
<td>[174]</td>
</tr>
<tr>
<td>Various&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Lkb1&lt;sup&gt;fl&lt;sup&gt;−&lt;/sup&gt;−&lt;/sup&gt;−; Pen&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Delayed tumor onset</td>
<td>[213]</td>
</tr>
</tbody>
</table>

<sup>a</sup> P53-deficient.

<sup>b</sup> Triple-negative.

<sup>c</sup> Erα-negative.

<sup>d</sup> Intestinal polyps, lymphomas, pheochromocytomas, and prostate, breast, and pancreatic carcinomas.

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AOM, azoxy methane; BOP, N-nitosobis-(2-oxopropyl)amine; NNK, 4-(methyleneamino)-1-(3-pyridyl)-1-butane (tobacco carcinogen); NML, N-methyl-N-nitrosourea.
inhibiting mitochondrial ATP production, thereby increasing the cellular AMP/ATP ratio. Also natural polyphenols such as berberine and resveratrol have been identified as indirect activators of AMPK [189,190]. AICAR is another indirect AMPK activator which, after uptake by the cells, is converted to ZMP, an AMP mimetic that binds to the AMPKα subunit [191,192]. Novel direct AMPK activators such as A-769662 [193] and PT1 [194] have been identified, of which A-769662 has been shown to target AMPKα subunit [195] while PT1 activates both α1 and α2 subunits by reducing its auto-inhibition [194]. Preclinical studies have shown that these AMPK activators can inhibit tumor cell growth [23,196–202].

### 4.2. Rapamycin

In 1999, rapamycin, a macroclide (Fig. 5b) was approved as an immuno-suppressant by the FDA, and used in organ transplantation to prevent allograft rejection [221]. Rapamycin specifically inhibits mTOR signaling by binding to the cytosolic FK-binding protein-12 (FKBP-12). This rapamycin–FKBP-12 complex binds to the mTOR protein resulting in the dissociation of mTORC1 (Fig. 5c) [222]. Because of the discovery of hyperactivated mTOR signaling in a variety of human cancers, rapamycin got attention for its putative efficacy in inhibiting cancer cell growth. Numerous studies have revealed that rapamycin inhibits growth of several cancer cell types such as breast, pancreas, prostate, kidney and lung in vitro, and reduces tumor growth and formation of metastases in tumor xenograft animal models [223–227]. Notably, rapamycin has been shown to increase the sensitivity for chemotherapy (gemcitabine) and radiotherapy in vitro and in vivo [228–231]. Additional in vivo studies demonstrated that treatment with rapamycin reduces tumor growth in different genetically engineered mouse models that spontaneously develop tumors (Table 4). In these models, mTOR signaling was activated by deletion of e.g. Lkb1, Tsc1 or Tsc2, Nf1, or Pten, but also by overexpression of Akt or Neu/ErbB2 (Table 4). Since these genetic events are common events in sporadic cancers as well as in cancers associated with hereditary hamartoma syndromes such as PJS, tuberous sclerosis complex (TSC), neurofibromatosis (NF) and Cowden’s Disease, rapamycin has been suggested to serve as an effective anti-tumor drug in these disorders [232].

Clinical trials have been set-up to test the efficacy of rapamycin in cancer patients, and in particular for patients with renal cell carcinoma (RCC), TSC or pancreatic neuroendocrine tumors (NETs). In an international randomized phase III trial, the efficacy of temsirolimus to treat RCC has been compared with conventional interferon (IFN) treatment (NCT00065468). Temsirolimus superiorly improved progression-free and overall survival in these patients [233]. A randomized placebo-controlled phase III trial documented that the use of everolimus in patients with advanced RCC after progression on sunitinib and/or sorafenib stabilized tumor progression, and improved progression-free survival with acceptable tolerability (RECORD-1, NCT00410124) [234,235]. In two nonrandomized, open-label trials (NCT00457808 and NCT00490789), it has been shown that sirolimus treatment of patients with TSC or sporadic lymphangioleiomyomatosis (LAM) resulted in regression of angiomyolipomas; however, these tumors tended to increase in volume after the therapy was stopped [236–238]. In a prospective, open-label phase I/II trial (NCT00411619), everolimus treatment of patients with subependymal giant cell astrocytomas (SEGA) was associated with marked reduction in tumor volume and seizure frequency [239]. A multicenter phase II trial (NCT00126672) showed that sirolimus treatment of TSC patients induced regression of kidney and liver angiomylipomas, and SEGAs [240]. Two phase II trials showed that everolimus, both alone and combined with octreotide long-acting release (LAR), improved disease control in patients with advanced NETs [241,242]. Two placebo-controlled phase III trials have been published investigating everolimus plus octreotide LAR versus octreotide LAR alone in patients with advanced carcinoids (RADIANT-2, NCT00412061) and everolimus monotherapy for advanced pancreatic NET (RADIANT-3, NCT00510068). In both studies everolimus improved progression-free survival with a low rate of severe adverse events [243,244]. Both temsirolimus (in May 2007) and everolimus (in March 2009) have been approved by the FDA for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib. In October 2010, the FDA approved the use of everolimus to treat subependymal giant cell astrocytomas in individuals with TSC who require treatment but are not candidates for surgery. In May 2011, everolimus has also been approved to treat patients with progressive NETs of the pancreas that are unresectable, locally advanced or metastatic.

Clinical studies to test the efficacy of mTOR inhibiting drugs in other cancers such as lung, breast, and CRC are currently ongoing. Besides the relatively high frequency of LKB1 mutations in sporadic NSCLC, mutations in EGFR and activation of AKT are frequently

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Genetically engineered tumor mouse models in which rapamycin treatment reduced tumor growth.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>Tumor type</td>
</tr>
<tr>
<td>Lkb1−/− mice</td>
<td>PJS-like gastrointestinal polyps</td>
</tr>
<tr>
<td>Apc+/− mice</td>
<td>Intestinal polyps</td>
</tr>
<tr>
<td>ApoC-III−/− mice</td>
<td>Intestinal polyps</td>
</tr>
<tr>
<td>Basal colon crypt cell-specific Apc−/− mice (Adeno-Cre)</td>
<td>Distal colon tumors</td>
</tr>
<tr>
<td>cis-NF1−/−:p53−/− mice</td>
<td>Malignant peripheral nerve sheath tumors</td>
</tr>
<tr>
<td>Tsc2−/− mice</td>
<td>Renal cystadenomas and liver hemangiomas</td>
</tr>
<tr>
<td>Neuro2A-treated Tsc2−/− mice</td>
<td>Renal cystadenomas</td>
</tr>
<tr>
<td>Pten−/− mice</td>
<td>Phaeochromocytomas and endometrial hyperplasia</td>
</tr>
<tr>
<td>Endometrial-specific Lkb1−/− mice (Sprf2-Cre)</td>
<td>Invasive endometrial carcinomas</td>
</tr>
<tr>
<td>MISsIR-TAG transgeneic mice</td>
<td>Ovarian tumors</td>
</tr>
<tr>
<td>Ovarian-specific Apc−/−:Pten−/− mice (Adeno-Cre)</td>
<td>Ovarian endometrioid adenocarcinomas</td>
</tr>
<tr>
<td>Pten−/−; Tgfα−/− mice</td>
<td>Follicular thyroid carcinomas</td>
</tr>
<tr>
<td>Bladder-specific Pten−/−; p53−/− mice (Adeno-Cre)</td>
<td>Invasive bladder carcinomas</td>
</tr>
<tr>
<td>Prostate-specific Pten−/−; p53−/− mice (Pb-Cre4)</td>
<td>Prostate tumors</td>
</tr>
<tr>
<td>Ph-Akt1 transgenic mice</td>
<td>Prostate tumors</td>
</tr>
<tr>
<td>MMTV-NeuYD transgenic mice</td>
<td>Mammary tumors</td>
</tr>
<tr>
<td>MMTV-NeuYD; VEGF transgenic mice</td>
<td>Mammary tumors</td>
</tr>
<tr>
<td>MMTV-c-Neu/ErbB2 transgenic mice</td>
<td>Mammary tumors</td>
</tr>
<tr>
<td>MMTV-PyMT transgenic mice</td>
<td>Gallbladder tumors</td>
</tr>
<tr>
<td>BCKS-ErbB2 transgenic mice</td>
<td>Gallbladder tumors</td>
</tr>
</tbody>
</table>

ENU, N-ethyl-N-nitrosourea; PJS, Peutz–Jeghers syndrome.

a Adeno-Cre delivery in distal colon.
b Adeno-Cre delivery in ovarian bursae.
c Adeno-Cre delivery in bladder lumen.
detected in these tumors as well [245,246]. Though all these events result in increased mTOR activity, the results of clinically inhibiting mTOR in NSCLC are so far not promising. Combined everolimus/gefitinib therapy in patients with advanced NSCLC led to a partial response in only 13% of the patients (8/62, of which two patients had mutations in KRAS) [247]. The LKB1 mutation status in these patients was not reported. As described in this review, loss of LKB1 is detected in breast carcinomas; however, loss of PTEN and mutations in PIK3CA has been detected more frequently. These genetic events all result in aberrant mTOR signaling, and are associated with resistance to hormonal therapy [248,249]. In a randomized placebo-controlled phase III trial (BOLERO-2, NCT00863655), postmenopausal women with hormone-receptor positive advanced breast tumors, resistant to endocrine therapy alone, were treated with everolimus and an aromatase inhibitor, which improved their progression-free survival [250]. This study indicates that inhibiting mTOR signaling sensitizes breast carcinomas to hormonal therapy in human patients. Also for advanced CRC, in which loss of LKB1 occurs infrequently but mTOR hyperactivation has been reported more commonly [251,252], inhibition of mTOR with rapamycin analogs has been proposed for reversion of chemotherapy resistance. Several phase I and phase II trials are currently investigating this hypothesis by adding everolimus or temsirolimus to chemotherapy treatment regimens (www.clinicaltrials.gov). Furthermore, results of a phase II trial for the treatment of refractory metastatic CRC in 50 patients with a combination of bevacizumab and sirolimus were published last year [253]. Unfortunately, the combination of drugs showed modest activity on the disease and considerable side effects.

In addition to the approved rapalogs sirolimus, temsirolimus and everolimus, the efficacy of a fourth rapalog, i.e. ridaforolimus, is currently being tested in phase III clinical trials. Besides these mTORC1 inhibitors, dual mTORC1 and mTORC2 inhibitors such as AZD8055 [140,254,255]; PP242 [256]; OSI-027 [244]; WAY-600; WYE-687; WYE-354 [257]; WYE-132 [258]; KU-0063794 [259]; and X-387 [260] have been developed and are currently being tested for their efficacy as anti-tumor agents.

4.3. Combining mTOR inhibitors with other specific pathway inhibitors

Rapamycin has been shown to be most effective in cells that rely on AKT signaling for their proliferation and tumor growth, whereas cells which depend on other signaling pathways for their growth are resistant to rapamycin [224,284,285]. Tumors that are responsive to rapamycin may develop resistance when alternative survival pathways, such as the mitogen-activated extracellular kinase (MAPK/ERK) signaling pathway, become activated [267,284]. Combination of rapamycin with other specific pathway inhibitors could solve this problem of resistance. Notably, rapamycin has been shown to synergize with specific inhibitors of e.g. the EGFR/ErbB2, MEK/ERK and Hedgehog signaling in vitro and in vivo [286–295].

In rapamycin-susceptible as well as rapamycin-resistant tumors, inhibition of mTOR by rapamycin treatment induces a feedback loop resulting in upregulation of PI3K/AKT signaling [296,297]. Therefore, blocking of both PI3K/AKT signaling and mTOR signaling by combining rapamycin with specific PI3K inhibitors (LY294002, Wortmannin, ZSTK474) has been shown to be more effective in tumor-growth inhibition than rapamycin alone [297–300]. Recently, a new inhibitor has been identified targeting both mTOR and PI3K, i.e. NVP-BEZ235 [301]. NVP-BEZ235 is an imidazo-quinoline derivative, which can bind to the ATP-binding cleft of both PI3K and mTOR [301]. Several in vitro and in vivo studies have shown that this dual inhibitor induces apoptosis and cell cycle arrest in a wide variety of tumor-cell types, and in most of these cases, the anti-tumor efficacy of NVP-BEZ235 was greater compared with that of rapamycin (e.g. [298,301–308]). Even in chemo-resistant tumor cells, or tumor cells which have been shown difficult to treat, dual PI3K/mTOR inhibition by NVP-BEZ235 efficiently inhibits tumor-cell growth, e.g. in KRAS-mutant NSCLC and in breast cancers resistant to ErbB2 inhibitors [309–314]. In addition, NVP-BEZ235 enhances sensitivity to chemotherapy and radiotherapy [315–318]. In some cases, combining NVP-BEZ235 with MEK or RAF inhibitors showed an increased efficacy in inhibiting tumor-cell growth, suggesting that some cancers require inhibition of both PI3K/mTOR and MEK/ERK signaling pathways for efficient anti-cancer treatment [296,319–322]. NVP-BEZ235 has entered phase I/II clinical studies for patients with advanced breast, renal, endometrial and other solid cancers (www.clinicaltrials.gov).

In addition to the NVP-BEZ235 inhibitor, novel dual PI3K/mTOR inhibitors such as PI-103 [323–325], PF-04691502 [326,327], GDC-0980 [328,329], NVP-BGTT226 [330–332], GSK2126458 [333], and PKI-402 [334,335] have been developed and are currently under investigation for their anti-tumor activities.

4.4. Other options to treat PJS-associated tumors

Since germ-line LKB1 mutations predispose to PJS, LKB1/AMPK/mTOR signaling has been proposed as a suitable target for treatment of PJS-associated hamartomas and carcinomas. In addition to the preclinical studies using metformin and rapamycin to inhibit tumor cell growth in vitro and in vivo as described in this review, other options for targeted treatment have been suggested. In tumors of Lkb1−/− mice as well as in tumors of PJS patients, elevated levels of cyclooxygenase-2 (COX-2) have been detected [336,337]. Inhibition of COX-2 with celecoxib, a non-steroidal anti-inflammatory drug, in Lkb1−/− mice reduced tumor burden and was associated with decreased vascularity [338]. In addition, 2 of 6 PJS patients responded well to celecoxib treatment as they showed reduced gastric polyposis [338], suggesting that inhibition of COX-2 serves a suitable strategy to treat PJS-associated lesions.

Recently, the SRC protein has been identified as a target of LKB1 in an integrative genomic and proteomic approach [339]. In this study, Src was shown to be activated in Lkb1-deficient Kras-mutated murine primary and metastatic lung tumors, which was validated in human lung carcinoma samples. Inhibition of Src by Dasatinib did not affect tumor growth, but it restored the sensitivity to PI3K/mTOR/MEK inhibition using NVP-BEZ235 and AZD2644 [339]. These results suggest that inhibition of SRC, whether or not in combination with other specific inhibitors, might be a suitable approach for treatment of PJS patients as well, although the activity status of SRC in PJS-associated lesions has to be determined yet.

5. Conclusion

As described in this review, loss of LKB1 is involved in a variety of human cancers, both in sporadic cancers and in cancers associated with PJS. LKB1 signaling has been attributed to a wide diversity of biological processes, involving a multitude of downstream mediators. These biological processes are known to be essential and deregulated in tumors, indicating the relevance of LKB1 loss in cancer. However, since only little is known about the biological consequences of LKB1 loss in carcinomas and the molecular mechanisms underlying these biological consequences, the exact tumor suppressor functions of LKB1 are yet to be elucidated. So far, most efforts to target LKB1 signaling in cancer have focused on activating AMPK and inhibiting mTOR. However, since activation of AMPK might require intact LKB1, and since it has been shown that some molecular and cellular responses of LKB1 loss are independent of AMPK and/or mTOR activity, targeting these two mediators might not be efficient in treating LKB1–associated cancer. More insight into the tumor suppressor functions and mediating molecular mechanisms of LKB1 will likely uncover additional and/or novel proteins and pathways to target, in order to treat LKB1–associated cancer. Nevertheless, hyperactivation of mTOR downstream of LKB1 is also mediated via other major signaling pathways such as the PI3K/AKT pathway.
survival pathway, which is frequently aberrantly activated in cancer. More insights into the genes and pathways deregulated in tumors and essential for their growth will provide novel strategies in order to develop therapeutic agents specifically targeting these pathways. In particular, a combination of these specific agents, and/or a combination of these agents with conventional hormonal, chemotherapy and radiotherapy are most likely to result in efficient anti-cancer regimes without inducing therapy resistance.

List of abbreviations
4E-BPs eukaryotic initiation factor 4E binding proteins
ACC acetyl CoA carboxylase
AMPK AMP-activated kinase
COX-2 cyclooxygenase-2
CRC colorectal cancer
DM2 Diabetes Mellitus type 2
FDA Food and Drugs Administration
FKBP-12 FK-binding protein-12
GI gastrointestinal
HIF-1α hypoxia-inducible factor 1 alpha
HSC hematopoietic stem cell
IFN interferon
IGF-1 insulin-like growth factor-1
IGF2R insulin-like growth factor 2 receptor
LAM lymphangioleiomyomatosis
LAR long-acting release
LKB1 liver kinase B1
LOH loss of heterozygosity
MEFs mouse embryonic fibroblasts
MO25 scaffolding mouse 25
NETs neuroendocrine tumors
NSCLC non-small cell lung carcinomas
P53 tumor suppressor protein
POCO polyoctylic ovarian syndrome
PKA cAMP-dependent kinase
PKCζ protein kinase C zeta
PTEN phosphatase and tensin homologue
RCC renal cell carcinoma
SEG A subependymal giant cell astrocytomas
Ser serine
STK11 serine/threonine kinase 11
STRAD STE20-related adaptor alpha
Thr threonine
TSC tuberous sclerosis complex

Conflict of interest
The authors do not have any conflicts of interest to declare.

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S. E. Korsse et al. / Biochimica et Biophysica Acta 1835 (2013) 194–210


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