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REVIEW

The long and winding road to rational treatment of cancer associated with LKB1/AMPK/TSC/mTORC1 signaling

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The liver kinase B1 (LKB1)/adenosine mono-phosphate-activated protein kinase (AMPK)/tuberous sclerosis complex (TSC)/mammalian target of rapamycin (mTOR) complex (mTORC1) cassette constitutes a canonical signaling pathway that integrates information on the metabolic and nutrient status and translates this into regulation of cell growth. Alterations in this pathway are associated with a wide variety of cancers and hereditary hamartoma syndromes, diseases in which hyperactivation of mTORC1 has been described. Specific mTORC1 inhibitors have been developed for clinical use, and these drugs have been anticipated to provide efficient treatment for these diseases. In the present review, we provide an overview of the metabolic LKB1/AMPK/TSC/mTORC1 pathway, describe how its aberrant signaling associates with cancer development, and indicate the difficulties encountered when biochemical data are extrapolated to provide avenues for rational treatment of disease when targeting this signaling pathway. A careful examination of preclinical and clinical studies performed with rapamycin or derivatives thereof shows that although results are encouraging, we are only half way in the long and winding road to design rationale treatment targeted at the LKB1/AMPK/TSC/mTORC1 pathway. Inherited cancer syndromes associated with this pathway such as the Peutz–Jeghers syndrome and TSC, provide perfect models to study the relationship between genetics and disease phenotype, and to delineate the complexities that underlie translation of biochemical and genetical information to clinical management, and thus provide important clues for devising novel rational medicine for cancerous diseases in general.
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The metabolic LKB1/AMPK/TSC/mTORC1 signaling pathway

The liver kinase B1 (LKB1)/adenosine mono phosphate activated protein kinase (AMPK)/tuberous sclerosis complex (TSC)/mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling pathway signaling pathway is central in regulating cellular metabolism and cell growth by integrating information regarding the intracellular energy and oxygen status, the presence of growth factors and nutrient availability. Under circumstances of sufficient energy and nutrient sources, this metabolic pathway stimulates cell growth. In cases of stress, metabolic processes are adjusted to restore resources in the cell.

LKB1/AMPK signaling

LKB1 (also referred to as serine/threonine kinase 11) is a 50 kDa serine/threonine kinase and is ubiquitously expressed in adult and fetal tissues, particularly in pancreas, liver, testes and skeletal muscle (Hemminki *et al*., 1998; Jenne *et al*., 1998). Lacking a nuclear export domain of its own, in the absence of stimulation, LKB1 is retained in the nucleus in an inactive state (Figure 1). Activation of LKB1 is associated with its translocation to the cytoplasm, which is induced upon formation of a heterotrimer with the STE20-related adaptor protein α (STRADα) and scaffolding mouse 25 protein (MO25) (Baas *et al*., 2003; Boudeau *et al*., 2003; Brajenovic *et al*., 2004) (Figure 1). By facilitating the binding of exportins to LKB1 and acting as a competitor for importin-α/β, STRADα prevents nuclear re-localization of LKB1 (Dorfman and Macara, 2008) (Figure 1). MO25 merely serves as a stabilizer of the LKB1-STRADα interaction (Boudeau *et al*., 2003). In addition to inducing its translocation, LKB1-STRADα interaction also results in LKB1 (auto) phosphorylation at various residues. However, the functional relevance of this remains debatable, as prevention of phosphorylation does not appear to affect LKB1 kinase activity (Jansen *et al*., 2009).

When activated, LKB1 phosphorylates and activates AMPKα and its related serine/threonine kinases (Figure 1) (Hawley *et al*., 2003; Lizcano *et al*., 2004; Shaw *et al*., 2004; Jaleel *et al*., 2005; Lim *et al*., 2010). Via the regulation of MARK isoforms and PAR proteins, LKB1 establishes cell polarity (Figure 1) (Spicer *et al*., 2003; Lizcano *et al*., 2004). AMPKα is

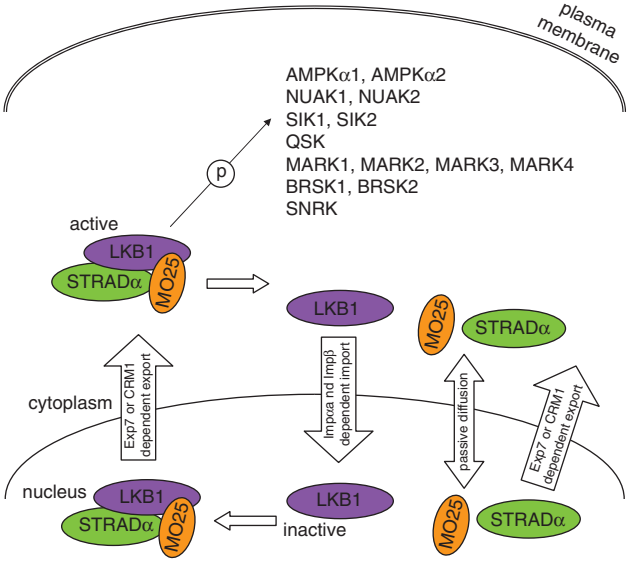

Figure 1 Activation and translocation of LKB1. LKB1 is activated by its translocation from the nucleus to the cytoplasm. Normally, LKB1 remains in the nucleus in an inactive state. Upon activation, LKB1 is bound by STE20-related adaptor protein α (STRADα) and mouse protein 25 (MO25), proteins that enter the nucleus either by passive diffusion or active import by importins α/β (Impα, Impβ). The stable LKB1/STRADα/MO25 complex is actively exported out of the nucleus by exportin 7 and CRM1. In the cytoplasm, LKB1 exerts its serine/threonine kinase activity by phosphorylating and activating the 14 members of the AMPK serine/threonine kinase family regulating cell polarity, energy metabolism and cell growth.

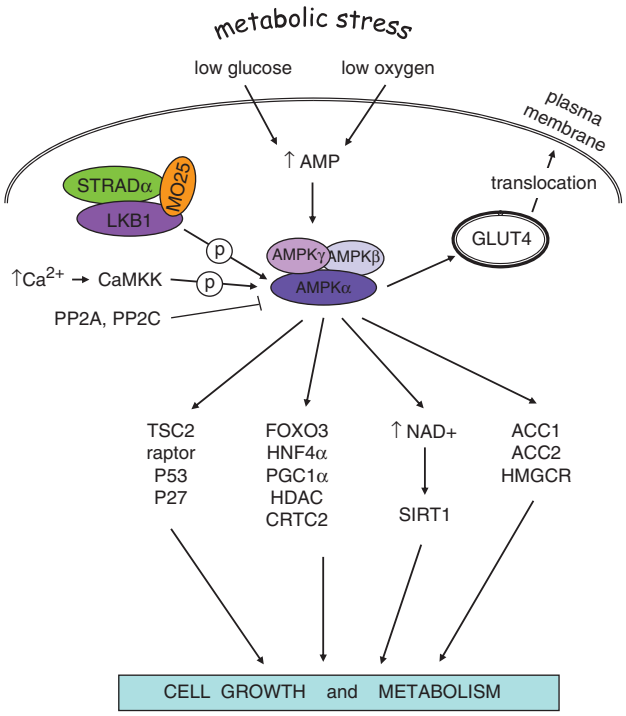

Figure 2 LKB1/AMPK signaling. The LKB1/AMPK signaling pathway regulates cell growth and metabolism via various downstream effectors. Metabolic stress induced by, for example, low glucose or oxygen levels increases intracellular AMP/ATP ratios. AMP is bound by the AMP-dependent protein kinase γ subunit (AMPKγ), which forms a complex with AMPKα and -β. Upon formation of this complex, AMPKα is phosphorylated by LKB1. AMPKα can also be phosphorylated by calcium/calmodulin-dependent protein kinase kinase (CaMKK) and is dephosphorylated by the protein phosphatases PP2A and PP2C. The activated AMPK complex induces translocation of the glucose importer GLUT4 to the plasma membrane to enhance glucose uptake and regulates downstream signaling controlling gene transcription and metabolic processes. Together, this results in the restoration of the energy balance in the cell.

activated by LKB1 in response to energy stress. When, due to either excessive ATP consumption or in the case of hypoxia, reduced aerobic ATP production, cellular AMP/ATP ratios are increased (Figure 2). This is sensed by AMPK, which through binding of AMP undergoes a conformational change, upon which it can be phosphorylated by LKB1 (Figure 2) (Alessi *et al*., 2006; Hardie, 2007; Jansen *et al*., 2009). AMPKα can also be phosphorylated by calcium/calmodulin-dependent protein kinase kinase, which is triggered through influx of calcium (Figure 2) (Hawley *et al*., 1995). The phosphorylation of AMPKα is reversed by the phosphatases PP2A and PP2C (Figure 2) (Moore *et al*., 1991).

LKB1/AMPK signaling induces several cellular processes, one of which is the control of energy metabolism through regulation of several downstream targets, including the metabolic enzymes acetyl-CoA carboxylase and HMG-CoA reductase (Figure 2) (Carling *et al*., 1987). By suppressing energy-consuming processes such as glycogen and lipid synthesis on the one hand, and enhancing energy-gaining pathways such as glycolysis on the other, AMPK activation aids in restoration of the cellular energy status (Kola *et al*., 2006). In line, AMPK activation induces relocalization of the glucose importer GLUT4 to the plasma membrane (Figure 2) (Kurth-Kraczek *et al*., 1999). In addition, LKB1/AMPK signaling regulates factors involved in cell cycle regulation, survival and gene transcription (Figure 2) (Jansen *et al*., 2009; Shackelford and Shaw, 2009). One of the downstream effectors of AMPK is TSC2 as described in more detail below. In conclusion, LKB1/AMPK signaling serves to coordinate energy metabolism, cell polarity and cell growth, processes which are all crucial in the development of cancer.

Regulation of the TSC1:TSC2 complex

The TSC1:TSC2 complex exists as a heterodimer of two proteins, the 130 kDa TSC1 (also referred to as hamartin) and the 200 kDa TSC2 (also referred to as tuberin) (Huang and Manning, 2008). TSC1 and TSC2 are widely expressed in human tissues, such as heart, brain, lung, liver, kidney, pancreas and skeletal muscle (Consortium, 1993; van Slegtenhorst *et al*., 1997; Huang and Manning, 2008). The two proteins interact through coiled-coil domains to form a stable, functional heterodimer (van Slegtenhorst *et al*., 1998). The C-terminal region of TSC2 shows homology to the Rap GTPase activating protein, and GTPase activating protein activity to various G-proteins (Wienecke *et al*., 1995; Xiao *et al*., 1997). In contrast, TSC1 does not have any

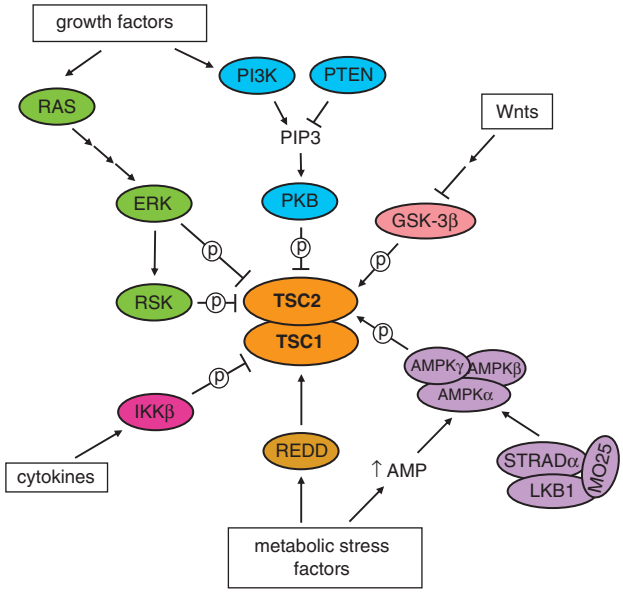

Figure 3 Regulation of the TSC1:TSC2 complex. The TSC1:TSC2 complex is regulated through various signaling pathways and serves as a nexus integrating information on intra and extracellular resources of growth, nutrient and metabolic factors. Upon metabolic stress, AMP-dependent activaton of AMPK by LKB1, results in phosphorylation and activation of TSC2. In hypoxic conditions, transcription of *REDD* is activated, which induces the TSC1:TSC2 complex. TSC2 is also activated by its phosphorylation by GSK3β, which is inhibited by Wnt-ligand activated Wnt signaling. Growth factor stimulation of its corresponding receptors activates both the RAS/ERK and PI3K/PKB pathways, which subsequently lead to the phosphorylation and inhibition of TSC2. Inhibition of the TSC1:TSC2 complex is also evoked by cytokine-induced phosphorylation by IKKβ.

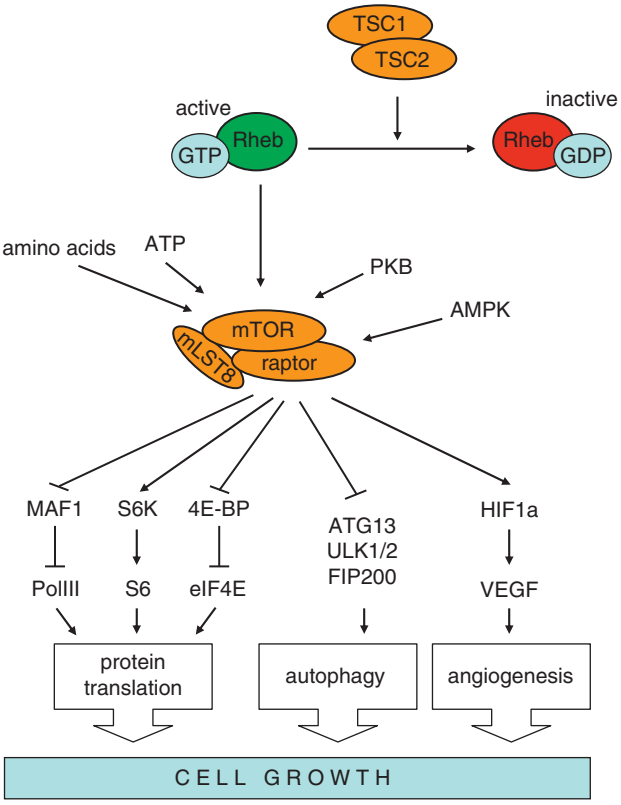

Figure 4 TSC/mTORC1 signaling. The mTOR complex 1 (mTORC1), consisting of mTOR, raptor and mLST8, controls cell growth mainly through the regulation of protein translation. Activated TSC1:TSC2 complex expresses GTPase activating protein activity towards Rheb, thereby inducing conversion of active GTP-bound Rheb to inactive GDP-bound Rheb. Active Rheb promotes mTORC1 activation, controlling protein translation by activating the ribosomal protein S6 kinase (S6K), inhibiting inhibition eukaryotic initiation factor 4E binding proteins (4E-BP) and inhibiting the RNA polymerase III (PolIII) repressor MAF1. In addition, mTORC1 induces angiogenesis through induction of hypoxia-inducible factor 1α (HIF1α)-dependent expression of vascular endothelial growth factor (VEGF), and inhibits autophagy by phosphorylating ATG13 and ULK1/2. In addition to TSC-dependent regulation of mTORC1 activity, the complex is directly activated by sufficient levels of energy (ATP) and nutrients (amino acids), as well as through phosphorylation of mTOR by PKB and of raptor by AMPK.

catalytic domains of its own but merely functions by preventing ubiquitin-mediated degradation of TSC2, thereby stabilizing intracellular TSC2 expression levels to maintain TSC2 activity (Benvenuto *et al*., 2000; Chong-Kopera *et al*., 2006).

Regulation of the TSC1:TSC2 complex is mainly achieved by phosphorylation. Although phosphorylation of TSC1 inhibits the complex, phosphorylation of TSC2 can either inhibit or activate TSC1:TSC2 activity (Figure 3) (Orlova and Crino, 2010). AMPK activates the TSC1:TSC2 complex by phosphorylating TSC2 on Thr1227 and Ser1345 in metabolic stress (Figure 3) (Inoki *et al*., 2003). Recently, the phosphorylation on Ser1345 has been shown to prime TSC2 for additional phosphorylation by GSK3β, which may be required for full activation of TSC2 (Inoki *et al*., 2006). As an alternative to AMPK-induced activation of the TSC1:TSC2 complex, hypoxia-induced metabolic stress causes increased transcription of *REDD*, which also results in TSC2 activation (Figure 3) (Sofer *et al*., 2005). In contrast to these stress-induced TSC1:TSC2 complex activation pathways, various growth factors and cytokines inhibit activation of the TSC1:TSC2 complex by inhibitory phosphorylation of TSC1 or TSC2 through PI3K/PKB (also known as AKT), ERK/RSK or IKKβ signaling modules (Figure 3) (Hay and Sonenberg, 2004; Inoki *et al*., 2005; Bhaskar and Hay, 2007; Huang and Manning, 2008). Phosphorylation of TSC2 by these kinases usually results in dissociation of the TSC1:TSC2 complex, followed by its degradation (Orlova and Crino, 2010). Thus, the TSC1:TSC2 complex integrates information on the available intra- and extracellular resources, and translates this into cell growth.

TSC/mTORC1 signaling

The activated TSC1:TSC2 complex regulates the activity of the mTORC1, a complex consisting of mTOR, raptor and mLST8. Therefore, the TSC1:TSC2 complex expresses GTPase activating protein activity towards Rheb, a small G-protein that promotes mTORC1 activity when GTP-bound (Figure 4) (Zhang *et al*.,

2003b). The activated TSC1:TSC2 complex induces conversion of active GTP-bound Rheb to inactive GDP-bound Rheb, which subsequently results in inhibition of mTORC1 (Figure 4) (Zhang *et al*., 2003b). In addition to the TSC1:TSC2-mediated inhibition of mTORC1, various metabolic factors such as ATP and amino acids regulate mTORC1 independent of the TSC1:TSC2 complex (Figure 4) (Dennis *et al*., 2001; Hay and Sonenberg, 2004). Furthermore, mTORC1 activity can be affected by direct phosphorylation of its components by several kinases that also directly phosphorylate TSC1:TSC2. For example, direct phosphorylation of mTOR by PKB, and of raptor by AMPK have been shown to regulate mTORC1 activity (Figure 4) (Vander Haar *et al*., 2007; Gwinn *et al*., 2008).

Upon activation, the mTORC1 controls cell growth by regulating 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) (Figure 4) (Hara *et al*., 2002; Kim *et al*., 2002). Recently, it has been shown that mTORC1 directly phosphorylates MAF1, thereby releasing its repressive action on RNA polymerase III leading to increased protein translation (Figure 4) (Kantidakis *et al*., 2010; Michels *et al*., 2010). In addition, activation of mTORC1 stimulates angiogenesis by inducing hypoxia-inducible factor 1α, which increases the expression of vascular endothelial growth factor (Figure 4) (Hudson *et al*., 2002). Furthermore, mTORC1 activity inhibits autophagy via phosphorylation of ATG13 and ULK1/2 (Figure 4) (Hosokawa *et al*., 2009; Jung *et al*., 2009). As these processes are all essential in tumorigenesis, it is not surprising to observe that deregulation of the LKB1/AMPK/TSC/mTORC1 signaling pathway is frequently associated with human cancer.

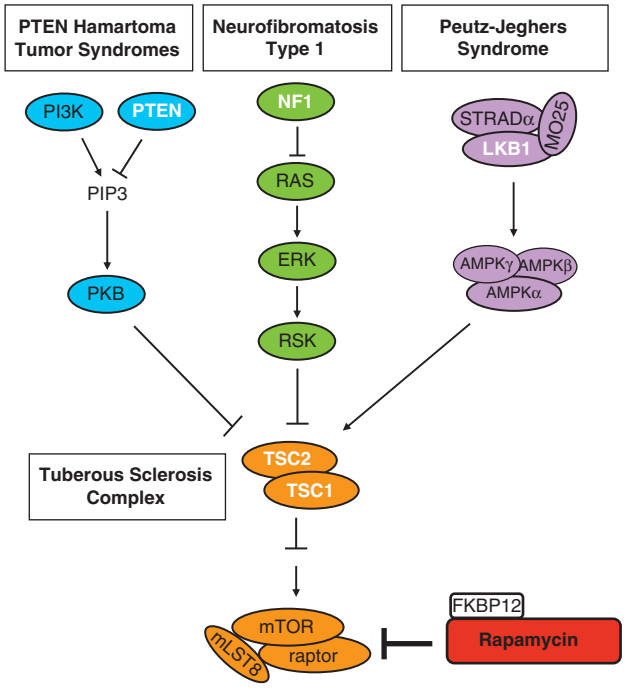

Figure 5 LKB1/AMPK/TSC/mTORC1 signaling in hamartoma syndromes. Genetic alterations in the LKB1/AMPK/TSC/mTORC1 pathway are involved in several hereditary hamartoma syndromes. Germ-line inactivation of the tumor suppressor *PTEN*, which normally inhibits PI3K/PKB signaling, predisposes to a variety of hamartoma syndromes grouped as PTEN hamartoma tumor syndromes. Germ-line inactivation of the tumor suppressor *NF1*, an inhibitor of the RAS/ERK pathway, predisposes to NF1. Germ-line inactivation of the tumor suppressor *LKB1*, the activator of AMPK, predisposes to the PJS. Germ-line inactivation of the tumor suppressors *TSC1* or *TSC2*, both predisposes to TSC. The genetic alterations associated with these hamartoma syndromes, all result in the inactivation of the TSC1:TSC2 complex causing impaired inhibition of mTORC1 leading to enhanced cell growth. Therefore, mTORC1 serves as an ideal target to be inhibited in order to treat patients suffering from these hamartoma syndromes. The pharmacological inhibitor rapamycin (and its analogs everolimus and temserolimus) forms an inhibitory complex with its intracellular receptor, FK506-binding protein (FKBP12), which binds mTOR thereby causing a dissociation and inhibition of mTORC1. Tumor suppressors are indicated in white.

LKB1/AMPK/TSC/mTORC1 signaling in disease

Through specific genetic alterations in different components of the LKB1/AMPK/TSC/mTORC1 signaling pathway, this metabolic pathway is associated with disease (Figure 5). Mutations in or loss of the tumor suppressors PTEN or neurofibromatosis type 1 (NF1) result in the activation of the PI3K/PKB or RAS/ERK signaling modules, respectively, which both subsequently inhibit the TSC1:TSC2 complex. In addition, inactivation of the tumor suppressor LKB1 leads to impaired activation of AMPK and the TSC1:TSC2 complex in metabolic stress conditions. Furthermore, the TSC1:TSC2 complex can be inactivated by mutations in its own encoding tumor suppressor genes. Together, these genetic alterations result in hyperactivation of the mTORC1-mediating downstream signaling, which induces cell growth.

Germ-line inactivation of the tumor suppressor genes *PTEN*, *NF1*, *LKB1*, *TSC1* and *TSC2* predisposes to a group of rare autosomal dominant inherited hamartoma syndromes (Figure 5). These hereditay disorders are characterized by the development of hamartomas in multiple tissues (Table 1). Hamartomatous polyps have a relatively benign appearance, but with a markedly disturbed architecture of cells present in the area in which they normally occur, that is, mesenchymal, stromal, endodermal, and ectodermal (Calva and Howe, 2008). 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 can be hyperplastic, and therefore, the malignant potential of hamartomas is still controversial. Despite the fact that these hamartomas follow a relatively benign course, they can cause, for example, bowel obstruction, seizures or hemorrhage, which may lead to severe complications and even death (Calva and Howe, 2008). In addition to the development

Table 1 Autosomal dominant inherited hamartoma syndromes associated with the metabolic LKB1/AMPK/TSC/mTORC1 signaling pathway

Disorder	*Prevalence*	*Gene (chromosome)*	*Hamartomatous lesions*	*Neoplastic lesions*	*Additional manifestations*
Tuberous sclerosis complex	1:5.800	*TSC1* (9q34) TSC2 (16p13.3)	(Sub) cortical tubers (glial harmartomas), subependymal glial nodules, retinal hamartomas, facial angiofibromas, (peri) ungual fibromas, renal angiomyolipomas, cardiac rhabdomyomas and pulmonary lymphangiomyomatosis.	Brain cancer (subependymal giant cell astrocytomas) and kidney cancer (cysts, oncocytomas, clear cell, papillary or chromophobe).	Hypomelanotic macules, forehead plagues, shagreen patches, developmental delays, epilepsy and autism.
Peutz–Jeghers syndrome	1:150.000	*LKB1* (*STK11*) (19p13.3)	Mainly in gastrointestinal tract (small intestines > stomach > colon/rectum), also in nose, bronchi, renal pelvis, uterus and bladder.	Mainly gastrointestinal cancer (colorectal, pancreatic, small intestinal, gastric and esophageal), also breast, lung, endometrial, ovarian (primarily granulosa cell subtype) and cervical (primarily highly malignant cervical adenoma malignum subtype) cancer. Rarely sex cord tumors with annular tubules.	Hyperpigmentation of lips, buccal mucosa, hands/feet, genitals, around nose/eyes.
PTEN hamartoma tumor syndrome[a]	1:200.000	*PTEN* (10q22-23)	Mucocutaneous lesions (facial trichilemmomas, acral keratoses, papillomatous papules) and intestinal hamartomas.	Breast, thyroid (follicular) and endometrial cancer.	Macrocephaly, developmental delays, mental retardation, scoliosis and hyperpigmentation genitals.
Neurofibromatosis type 1	1:3.500	*NF1* (17q11.2)	Plexiform and cutaneous neurofibromas, schwannomas, iris hamartomas (Lisch nodules).	Malignant peripheral nerve sheet tumors, gliomas and astrocytomas.	Café au lait spots, axillary freckles, scoliosis, cognitive and learning disabilities, skeletal abnormalities.

Abbreviations: AMPK, adenosine mono-phosphate-activated protein kinase; LKB1, liver kinase B1; mTORC1 mammalian target of rapamycin complex 1; TSC, tuberous sclerosis complex.
[a]PTEN hamartoma tumor syndrome include Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, proteus syndrome and Lhermitte–Duclos disease.

of multiple hamartomas, these polyposis syndromes are associated with the development of a variety of cancers as well. In addition, somatic alterations in genes involved in the LKB1/AMPK/TSC/mTORC1 pathway have been associated with a wide variety of sporadic human cancers.

Tumor suppressor gene LKB1 *and cancer*

Inactivating *LKB1* mutations are detected in 5–17% of sporadic non-small cell lung carcinomas and in 5% of pancreatic cancers and melanomas. Promoter hypermethylation or loss of LKB1 expression has been described for sporadic testicular, papillary breast, endometrial, neuroendrocrine lung and pancreatic cancer (Hezel and Bardeesy, 2008). Germ-line mutations in *LKB1* predispose to the Peutz–Jeghers syndrome (PJS), (Hemminki *et al*., 1998; Jenne *et al*., 1998) which is characterized by mucocutaneous hyperpigmentation, gastrointestinal hamartomatous polyposis and a highly increased risk for developing gastrointestinal, breast, gynecological and lung cancer (Tomlinson and Houlston, 1997; McGarrity and Amos, 2006; van Lier *et al*., 2010) (Table 1).

LKB1 is classified as a tumor suppressor gene implying that both alleles need to be inactivated to induce tumor development. Loss of heterozygosity of the remaining *LKB1* allele has been detected in a subset of PJS-hamartomas, however, it is observed more frequently in carcinomas. Around 150 different mutations without a hotspot in *LKB1* have been associated with PJS, without a clear genotype-phenotype correlation, have been detected (Yoo *et al*., 2002). The majority of mutations results in truncation or abnormal splicing, although in $\sim$20% of the cases, a missense mutation in the kinase domain of *LKB1* is detected. The truncating mutations tend to associate with an earlier age of onset of disease as compared with PJS cases associated with missense mutations in *LKB1* (Yoo *et al*., 2002). The effect of most PJS-associated missense mutations on LKB1 function has not yet been investigated.

To investigate the tumor suppressor function of LKB1, mouse models have been generated and characterized. Homozygous loss of *Lkb1* is embryonically lethal, whereas mice with a heterozygous deletion are tumor prone, showing an increased incidence of spontaneous tumor formation as well as increased susceptibility to toxicity-induced carcinogenesis (Ylikorkala *et al*., 2001; Jishage *et al*., 2002; Miyoshi *et al*., 2002; Gurumurthy *et al*., 2008). Moreover, *Lkb1*+/− mice develop hamartomatous polyps in the stomach and intestines, but, similar to their human counterparts, these polyps appear to lack or have only low malignant potential (Jishage *et al*., 2002; Miyoshi *et al*., 2002). Interestingly, heterozygous loss of *Lkb1* in

myofibroblasts has been shown to be sufficient to induce hamartoma formation in mice, indicating that these stromal cells are the driving force of hamartomas, and that LKB1 can function as a haploinsufficient tumor suppressor (Katajisto *et al*., 2008). Finally, conditional *Lkb1* loss in various tissues is also associated with the development of cancer (Contreras *et al*., 2008; Hezel *et al*., 2008; Pearson *et al*., 2008; McCarthy *et al*., 2009).

In conclusion, the phenotypes observed in human patients and mouse models indicate that loss of LKB1 activity is involved in the development of a variety of cancers.

Tumor suppressor genes TSC1 *and* TSC2 *and cancer*

Inactivating mutations in *TSC1* and *TSC2* are detected mainly in sporadic renal cell carcinomas (RCCs) (Knowles *et al*., 2003). Loss of chromosome 16p (locus for *TSC2*) or promoter hypermethylation of the *TSC* genes has been detected in a substantial proportion of ovarian, gall bladder and breast cancer (Knowles *et al*., 2003; Jiang *et al*., 2005). Germ-line mutations in *TSC1* and *TSC2* predispose to TSC (Consortium, 1993; van Slegtenhorst *et al*., 1997), which is characterized by the development of widespread hamartomas in several organs including brain (cortical tubers and subependymal glial nodules), kidneys (angiomyolipomas, AML), skin ((angio) fibromas), heart (rhabdomyomas) and lungs (lymphangioleiomyomatosis (LAM)) (Table 1). In addition, these patients develop early onset brain cancer (subependymal giant cell astrocytomas (SEGAs)) and different types of renal cancer (Table 1) (Schwartz *et al*., 2007; Curatolo *et al*., 2008; Ess, 2010). Notably, LAM also occurs sporadically, owing to somatic mutations in the *TSC* genes, and is associated with renal AML in $\sim$50% of these cases (Carsillo *et al*., 2000).

Both *TSC* genes are characterized as tumor suppressor genes and loss of function of either gene leads to hamartoma formation. Loss of heterozygosity in affected organs is frequently detected in combination with *TSC2* mutations, but only rarely with mutations in *TSC1*. Genetic studies have revealed large deletions and missense mutations in *TSC2*, whereas the majority of mutations in *TSC1* are small and result in expression of a truncated protein. No mutational hotspots in either *TSC1* or *TSC2* have been identified. In fact, over 300 different mutations have been described for *TSC1*, and even over a 1000 for *TSC2*, probably contributing to the clinical variability observed among TSC patients. Although a clear genotype-phenotype correlation has not been identified for TSC, *TSC2* mutations are associated with a more severe disease phenotype compared with *TSC1* mutations (Sancak *et al*., 2005).

The tumor suppressor functions of TSC1 and TSC2 have been studied using animal models. In mice, homozygous loss of either *Tsc1* or *Tsc2* result in embryonic lethality, whereas in heterozygous animals increased tumor formation including renal cystadenomas, and learning deficits are apparent (Rennebeck *et al*., 1998; Kobayashi *et al*., 1999; Kwiatkowski *et al*., 2002). Additionally, epilepsy, brain malformations, RCCs and additional tumors have been described in Eker rats, which have a naturally occurring inactivating *Tsc2* mutation (Cook and Walker, 2004; Hino, 2004; Yeung, 2004). Epilepsy and brain abnormalities were also detected in mice with a specific *Tsc1* deletion in cells of the neuronal system (Uhlmann *et al*., 2002).

Together, these observations indicate that loss of a functional TSC1:TSC2 complex contributes to the development of a variety of benign and malignant tumors.

Genetic alterations in other components of the LKB1/AMPK/TSC pathway and cancer

PI3K/PKB signaling, which is counteracted by the lipid phosphatase PTEN, modulates LKB1/AMPK/TSC/mTORC1 signaling and activates the mTORC1 directly or via inhibiting the TSC1:TSC2 complex (Figures 3 and 4). Thus, activation of the PI3K/PKB signaling cassette by genetic alterations in *PTEN*, *PIK3CA* or *PKB* result in activation of mTORC1 signaling and is associated in different types of human cancer (Sansal and Sellers, 2004). Somatic inactivating mutations in the tumor suppressor gene *PTEN* are frequently detected in glioblastomas, melanomas, and in prostate and endometrial cancers (Sansal and Sellers, 2004). Loss of PTEN expression has been observed in sporadic hepatocellular carcinomas (Villanueva *et al*., 2008).

In addition to inactivation of PTEN, activation of PI3K or PKB is observed in several types of sporadic human cancer. Activation of PI3K has been described for ovarian, gastric, head and neck squamous cell carcinomas (by amplification of *PIK3CA*), and for gastric, colorectal and mammary carcinomas, and glioblastomas (by somatic missense mutations in *PIK3CA*) (Altomare and Testa, 2005). Amplification, overexpression or activation of PKB has been observed in ovarian, pancreatic, hepatocellular, mammary, prostate and colorectal carcinomas (Altomare and Testa, 2005). In neuroendocrine tumors (NETs), derived from neuroendocrine cells scattered throughout the body, for example, in the pancreas, stomach, (para) thyroid and pituitary glands, overexpression of PKB and decreased expression of TSC2 and PTEN has been observed (Dworakowska and Grossman, 2009; Missiaglia *et al*., 2010). NETs have even been suggested to be a clinical feature of TSC (Dworakowska and Grossman, 2009).

Inactivating germ-line mutations in *PTEN* predispose to a variety of different hereditary syndromes, such as Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, proteus syndrome and Lhermitte–Duclos disease, which have been proposed to be grouped together as PTEN hamartoma tumor syndromes (PHTS), all characterized by the formation of hamartomas and cancerous lesions (Table 1) (Sansal and Sellers, 2004).

Another modifier of the LKB1/AMPK/TSC/mTORC1 pathway, which is also associated with hamartoma and cancer development is NF1. *NF1* encodes neurofibromin, which activates Ras-GTPase. Accordingly, in NF1-deficient tumors, Ras is hyperactivated, which, in addition to other downstream

pathways, activates mTORC1 signaling. Germ-line inactivation of *NF1* results in NF1, which is characterized by the development of benign (neurofibromas) and malignant peripheral nerve sheath tumors and hamartomatous lesions of the iris (Table 1) (Parrinello and Lloyd, 2009).

The LKB1/AMPK/TSC module constitutes a canonical signal transduction pathway controlling mTORC1 activity whose importance for the integration of cellular nutritional status and survival signaling to external cues is undisputed. However, the disease phenotypes related to genetic alterations within this pathway are highly heterogeneous, and show no overlap. The knowledge on molecular biology and biochemistry has provided us with a linear view on signaling pathways and the responses due to alterations in these pathways. On the other hand, these genetic disorders show the importance of external modifiers of the pathway for final clinical outcome in the context of complex systems like living organisms. Why aberrant LKB1/AMPK/TSC signaling results in these highly tissue-specific disease phenotypes is not yet completely understood, but may be due to the high level of biological robustness of the pathway. For example, it induces feed-forward inhibition through the transcription of miR451, which in turn downregulates the activity of the entire cassette (Godlewski *et al*., 2010). In addition, signaling thresholds, the presence of redundant enzymes and feed-back mechanisms lead to a highly complex system of biochemical interaction whose outcome shows a great deal of cell type and context-dependent specificity.

Hyperactive mTORC1 in LKB1/AMPK/TSC-associated lesions

As described above, various lesions are associated with alterations in the metabolic LKB1/AMPK/TSC signaling pathway. As inactivation of this signaling cassette impairs the inhibition of mTORC1, it is anticipated that lesions associated with LKB1/AMPK/TSC inactivation show enhanced mTORC1 activity. The activation status of mTORC1 is commonly determined by the phosphorylation of its downstream effectors S6K, the ribosomal protein S6 and/or 4E-BP (Figure 4).

The mTorc1 hyperactivation has indeed been observed in *Lkb1*-null mouse embryonic fibroblasts as well as in other murine and human *LKB1*-deficient cells (Corradetti *et al*., 2004; Shaw *et al*., 2004, 2005; Carretero *et al*., 2007; Contreras *et al*., 2008; Ikeda *et al*., 2009). In addition, mTorc1 hyperactivation has been observed in intestinal polyps of *Lkb1*+/− mice and PJS patients, indicating that mTORC1 is hyperactivated in PJS-associated hamartomas (Shaw *et al*., 2004; Shackelford and Shaw, 2009). Whether mTORC1 is hyperactivated in PJS-associated carcinomatous lesions as well, is yet to be determined.

In *Tsc1*- and *Tsc2*-null mouse embryonic fibroblasts as well as in other *Tsc*-deficient cells, increased mTorc1 activity has been detected (Kwiatkowski *et al*., 2002; Onda *et al*., 2002; Zhang *et al*., 2003a; Uhlmann *et al*., 2004; Habib *et al*., 2010). In skin fibroblasts of TSC patients, and in *TSC2*-deficient AML cells from a patient with LAM, mTORC1 hyperactivation has been detected (Jozwiak *et al*., 2009; Lee *et al*., 2010). In addition, in renal cystadenomas from *Tsc1*- and *Tsc2*-deficient mice (Kwiatkowski *et al*., 2002; Zhang *et al*., 2003a), as well as in surgically resected tubers, SEGAs and AMLs from TSC patients, increased levels of phospho-S6 could be detected, indicating that mTORC1 is hyperactivated in TSC-associated lesions (El-Hashemite *et al*., 2003; Baybis *et al*., 2004; Chan *et al*., 2004; Miyata *et al*., 2004).

Additionally, downstream mTORC1 signaling has been shown to be active in *Nf1*- and *Pten*-deficient cells (Podsypanina *et al*., 2001; Johannessen *et al*., 2008). Hyperactivation of mTORC1 have further been established for RCC cell lines and tumors as well as for various NET cell lines and animal models (Chan *et al*., 2010), and in ~50% of hepatocellular carcinomas (Villanueva *et al*., 2008). Together, the observed exaggerated activation of mTORC1 in these LKB1/AMPK/TSC-associated lesions suggests that mTORC1 would serve an effective target for therapy to treat these cancerous and hamartomatous lesions.

Treatment of LKB1/AMPK/TSC-associated lesions with mTORC1 inhibitors

Rapamycin and analogs as anticancer drugs

Rapamycin, also known as sirolimus, is a macrolide antibiotic first discovered in the 1970's on Easter Island as a product of the bacterium *Streptomyces hygroscopicus* (Sehgal *et al*., 1975; Vezina *et al*., 1975), and has been identified as an effective inhibitor of mTORC1 (Heitman *et al*., 1991). Rapamycin forms an inhibitory complex with the cytosolic FK-binding protein-12. Binding of this complex to the mTOR protein results in the dissociation of mTORC1, thereby inhibiting its ability to phosphorylate downstream substrates (Figure 5) (Chung *et al*., 1992). Due to its ability to inhibit T- and B-cell proliferation and activation (Dumont *et al*., 1990; Wicker *et al*., 1990), rapamycin and its analogs RAD001 (everolimus) and CCI-779 (temsirolimus) have been approved by the Food and Drug Administration (FDA) as immunosuppressive agents in the United States, and is now commercially available. In addition, these drugs appear to affect tumor growth by inducing tumor cell apoptosis and suppressing angiogenesis (Law, 2005). Temsirolimus (in May 2007) and everolimus (in March 2009) have been FDA approved for the treatment of advanced RCC after failure of first-line treatment with sunitinib or sorafenib. Only very recently, in October 2010, the FDA approved the use of everolimus also for the treatment of TSC-associated SEGAs that can not be surgically removed. Currently, several phase III trials testing the efficacy of rapamycin and its analogs are ongoing for a variety of malignancies such as breast, gastric and hepatocellular cancer, mantle cell

lymphoma and cancers associated with transplantation (http://www.clinicaltrials.gov). Preclinical studies show that everolimus is able to inhibit proliferation of several NET cell lines, and ongoing clinical trials investigating the use of everolimus for the treatment of sporadic NETs provide evidence that the drug is able to reduce tumor size and control disease progression (Chan *et al*., 2010).

Thus, rapamycin analogs are approved anticancer drugs, and their efficacy in the treatment of a variety of sporadic cancers is currently being tested.

The mTORC1 inhibitors as treatment for hamartoma syndromes

Previously, it has been shown that rapamycin is able to inhibit mTorc1 signaling in *Lkb1*-deficient cells (Shaw *et al*., 2004). Preclinical evaluation of the suppressive and preventive efficacy of rapamycin for PJS using *Lkb1*+/− mice has revealed that rapamycin effectively reduced the tumor burden in these animals (Wei *et al*., 2008, 2009; Robinson *et al*., 2009; Shackelford *et al*., 2009). Also in mouse models for NF1 and PHTS, treatment with rapamycin has been shown to reduce tumor growth (Podsypanina *et al*., 2001; Hegedus *et al*., 2008; Squarize *et al*., 2008). At present, one open-label phase II clinical trial is recruiting PJS patients for suppressive therapy with everolimus to determine if this drug can diminish gastrointestinal polyps (clinicaltrials.gov identifier NCT00811590). Another phase II trial recruiting PJS patients with advanced cancer is currently active (clinicaltrials.gov identifier NCT01178151). Four clinical phase I/II trials are ongoing including NF1 patients to test the safety and efficacy of rapamycin and everolimus to treat gliomas, cutaneous fibromas and neurofibromas in these patients (clinicaltrials.gov identifiers NCT01158651, NCT01031901, NCT00634270, NCT00652990). To test the efficacy of rapamycin to treat patients with Cowden syndrome and other PTEN-associated syndromes, one clinical phase II trial is currently ongoing (clinicaltrials.gov identifier NCT00971789). Preclinical and clinical studies evaluating the efficacy of rapamycin and analogs for the treatment of lesions associated with TSC are described in more detail below.

The mTORC1 inhibitors as treatment for TSC

Preclinical studies have demonstrated the ability of rapamycin to inhibit mTORC1 activity and cell growth in murine and human *TSC1*- or *TSC2*-deficient cells (Kwiatkowski *et al*., 2002; Onda *et al*., 2002; Zhang *et al*., 2003a; Uhlmann *et al*., 2004; Jozwiak *et al*., 2009; Mi *et al*., 2009; Habib *et al*., 2010; Lee *et al*., 2010). *In vivo*, rapamycin treatment reduced the tumor burden and increased survival in *TSC*-deficient animal models developing renal tumors (Kenerson *et al*., 2005; Lee *et al*., 2005, 2006; Pollizzi *et al*., 2009; Woodrum *et al*., 2010).

The first case report of rapamycin-therapy for TSC-associated tumors in patients has been published in 2006 (Franz *et al*., 2006). Four TSC-patients with SEGAs and one with a pilocytic astrocytoma were treated with oral rapamycin at standard immunosuppressive doses (serum levels 5–15 ng/ml) for 2.5–20 months. During follow-up, neuroimaging showed regression of all lesions, and necrosis could be observed in one case. Known side effects of rapamycin such as aphthous ulcers, acneiform rash and elevation of serum cholesterol were reported. In one patient, discontinuation of therapy led to the increase of SEGAs, and subsequent regression after reintroduction of rapamycin. Since then, a number of additional case reports have been published on this topic (Table 2).

The results of the first formal prospective phase I/II open-label clinical trial of sirolimus therapy for patients with TSC or sporadic LAM, in which both are associated with AML development, were published in 2008 (Bissler *et al*., 2008). A cohort of 25 adult patients, 19 with TSC (of who 12 had LAM) and 6 with sporadic LAM, were treated with sirolimus (5–15 ng/ml) for 1 year, followed by a 12-month follow-up period. Evaluation after 1 year of therapy showed a reduction in AML volume of at least 30% in 16 of 20 patients (including 14 TSC patients). However, during the year of follow-up after cessation of therapy, AML regrowth occurred in 17 of 18 patients completing the trial. Furthermore, effects on pulmonary function were elusive, and no effect on cortical tuber size could be detected. Simultaneously, interim findings of another, ongoing phase II trial were revealed (Table 3, clinicaltrials.gov identifier NCT00490789) (Davies *et al*., 2008). Although a decrease of AML size could be detected in all included patients, no significant improvement in lung function was observed. In both trials, a high rate of adverse events of sirolimus therapy was reported, though they were mostly low-grade and self-limiting.

Recently, the results of an open-label study investigating the use of everolimus for SEGAs in young TSC patients (age $\geqslant$3 years) have been published (Krueger *et al*., 2010). Twenty-eight patients (median age 11 years, 22 under 18 years of age) had been treated with everolimus at a dose of 3.0 mg/m^2. In the majority of patients (75%), therapy was associated with a clinically meaningful reduction in SEGA volume of at least 30% at 6 months. The decrease in tumor volume was most obvious during the initial 3 months of treatment after which a sustained response could be observed. In one patient, after regression of SEGA volume at 6 months of therapy, progression of tumor volume had been detected. All patients included in this study had at least one adverse event during everolimus therapy, of which four were of serious nature. At present, a number of formal clinical trials of mTORC1 inhibitors for the treatment of TSC-associated manifestations are ongoing (Table 3) (clinicaltrials.gov).

Together, this indicates that in addition to RCC, and TSC-associated SEGAs, rapamycin treatment may be beneficial for other LKB1/AMPK/TSC/mTORC1-associated sporadic cancers and hereditary hamartoma syndromes as well. However, several concerns can be raised for rapamycin as an anticancer drug as can be learned from the studies described above. Although promising effects for sporadic and TSC-associated renal

Table 2 Case reports of mTORC1 inhibitors for the treatment of TSC-associated lesions

Reference	*Baseline characteris-tics*	*Indication*	*Daily dose (serum levels)/ duration of treatment/ intercurrent therapy*	*Adverse events*	*Response*
Franz *et al.* (2006)	Case 1: female (21 years). Case 2: female (15 years). Case 3: female (3 y ears). Case 4: male (5 years). Case 5: male (14 years)	Case 1: bilateral SEGAs. Case 2: SEGA. Case 3: low-grade pilocytic astrocytoma with hydrocephalus. Case 4: SEGA. Case 5: SEGA	Case 1: rapamcyin 6 mg (7.7 ng/ml). Case 2: rapamycin 7 mg (10.9 ng/ml)/lamotrigine, phenobarbital. Case 3: rapamycin 4 mg (10.2 ng/ml). Case 4: rapamycin 5 mg (9.6 ng/ml)/divalproex sodium, clonidine, quetiapine and amitriptyline. Case 5: rapamycin 6 mg (10.4 ng/ml)	Case 1: Aphthous ulcers, acneiform rash and serum cholesterol elevation (self-resolved). Case 2: serum cholesterol elevation. Case 3: transient hypercholesterolemia. Case 4: none. Case 5: acneiform rash, oral aphthous ulcers and transient serum cholesterol elevation	Case 1: decreased SEGA size at 2.5–5 months; stopped at 5 months; size re-increased 4 months after stopping; decreased 4–20 months after resuming. Case 2: decreased SEGA size and seizure-free at 5 months. Case 3: Decreased ventriculomegaly with SEGA necrosis at 5 weeks; resolved ventriculomegaly with decreased SEGA size and necrosis at 3.5–6 months; remained seizure-free; progress in speech and rehabilitative therapies. Case 4: decreased SEGA size at 3 months; seizure control. Case 5: decreased SEGA size at 2.5 months.
Wienecke *et al.* (2006)	Male (19y ears)	AMLs, multiple cerebral tubers and subependymal nodules	Rapamycin 2 mg (4–5 ng/ml)/6 months	Acneiform skin rash	Significant decrease in tumor size of one AML during treatment, increase after 8 months without treatment, decrease after readministration. No change of other AML and cerebral manifestations.
Herry *et al.* (2007)	Female (38 years)	Bilateral AMLs	Rapamycin 6 mg (8–10 ng/ml)/2 years	None	Reduction of AMLs size within the first year of therapy, stabilization thereafter.
Corsenca *et al.* (2007) Hofbauer *et al.* (2008)	Female (21 years)	Immunosuppressive therapy after TSC-associated renal transplantation	3–5 mg/mycophenolate mofetil, prednisone	None	No progression of subependymal nodules and cortical tubers and GFR improvement at 6 months FU. Improvement of facial angiofibromas, no change in ash leaf-like hypopigmented macules.
Koenig *et al.* (2008)	Female (21 years)	Bilateral SEGAs	Rapamycin 0.2 mg/kg (11–13 ng/ml)/5 months	None	Decreased SEGA size at 2.5 months.
Muncy *et al.* (2009)	Female (10 years)	Seizure control	Rapamycin 0.15 mg/kg (9.8 ng/ml)/topiramate, oxcarbamazepine	Skin breakdown, mouth ulcers, and frequent viral infections at dose 0.2 mg/kg per day	Reduction in seizure frequency. No change in number or size of cortical tubers.
Lam *et al.* (2010)	Case 1: male (9 years). Case 2: male (13 years). Case 3: female (10 years)	SEGAs	Case 1: rapamycin 7 mg (10–15 ng/ml)/1 year. Case 2: rapamycin 6 mg (14 ng/ml)/NR. Case 3: rapamycin 9 mg (8.4 ng/ml)/NR	Case 1: oral ulcers, myalgias, transient hypercholesterolemia and gingival hypertrophy. Case 2: mouth sores and transient hypercholesterolemia. Case 3: mouth sores.	Case 1: 65%; SEGA decrease after 3 month FU, unchanged at 6 and 9 months. Case 2: % SEGA decrease after 3 months FU. Case 3: 50% SEGA decrease after 3 months FU.
Tarasewicz *et al.* (2009)	Female (44 years)	Immunosuppressieve therapy after TSC-associated renal transplantation	Rapamycin 2 mg/2 years/ methylprednisolone, tacrolimus	None	Improvement of skin lesions, no change in cystic lesions in pulmonary parenchyma, more cortical tubers.
Haidinger *et al.* (2010)	Case 1: male (35 years). Case 2: female (40 years). Case 3: female (51 years)	Immunosuppressive therapy after TSC-associated renal transplantation	Rapamycin	Case 1: diarrhea, urinary tract infections and hyperlipidaemia. Case 2: none. Case 3: diarrhoea	Case 1: stabilization of brain lesions after 18 months FU, stable allograft function, GFR improvement and decrease of size and erythema of facial angiofibromas after 24 months FU. Case 2: improvement of pulmonary function parameters and no growth of astrocytomas after 12 months FU. Case 3: stable CNS morphology, no evidence of tumor growth, stable renal function, skin lesions unchanged after 24 months FU.

Table 2 Continued

Reference	*Baseline characteris-tics*	*Indication*	*Daily dose (serum levels)/ duration of treatment/ intercurrent therapy*	*Adverse events*	*Response*
Pressey *et al.* (2010)	Male (7 years)	Recurrent chest wall fibromatosis and multifocal RCC	Rapamycing 7.2 mg/m^2 (9–12 ng/ml)/22 months	Minimal	Regression of fibromatosis tumor and RCC tumors in the first 9 months of therapy, stabilization during the subsequent 13 months of therapy.
Krischock *et al.* (2010)	Female (7 years)	Multiple AMLs in a solitary kidney	Rapamycin 1.9 mg/m^2 (3–6 ng/ml)/NR	Serum cholesterol elevation	AML number decreased at 6 months after starting treatment, stabilization thereafter. Facial angiofibromas smaller, paler and less itchy.
Sparagana *et al.* (2010)	Female (12 years)	Bilateral SEGAs, right retro-ocular optic nerve tumor.	Rapamycin 5 mg (8–12 ng/ml)/16 months	NR	49% decrease in tumor volume of largest SEGA. After initial reduction 6% increase of right optic nerve tumor volume.
Haemel *et al.* (2010)	Female (16 years)	Facial angio-fibromas	1% topical rapamycin twice daily (< 2 ng/ml)/ 3 months	None	Decreased erythema, reduced number of angiofibromas, vascular papules smaller or resolved.
Peces *et al.* (2010)	Male (40 years)	Bilateral multiple AMLs	Rapamycin 1 mg (1–4 ng/ml)/1 years	None	17% reduction of total kidney volume, improvement of renal function and facial angiofibromas at 12 months.
Kaufman McNamara *et al.* (2010)	Case 1: male (21 years). Case 2: male (6 years)	Facial angio-fibromas	Case 1: topical rapamycin 60 ml 1 mg/ml solution, after 5 months oral sirolimus 3 mg/ 8 months. Case 2: topical rapamycin 60 ml 1 mg/ml solution/ 3 months	None	Case 1: improvement in numbers of angiofibromas and erythema. Case 2: complete resolution of all lesions and associated erythema.
Yalon *et al.* (2010)	Male (28 years)	Multiple SEGAs	Everolimus 10 mg/11 months	Bilateral edema of the hands. Elevated CPK and hypertension.	Regression of SEGAs after 3, 6 and 11 months of treatment. Stabilization of SEGAs after 10 months discontinuation of treatment.

Abbreviations: AML, angiomyolipomas; CPK, creatine phosphokinase; FU, follow-up; GFR, glomerular filtration rate; NR, not reported; RCC, renal cell carcinoma; SEGA, subependymal giant cell astrocytoma; TSC, tuberous sclerosis complex.

AMLs and cutaneous angiofibromas are observed, after initial reduction of lesion sizes, the disease stabilizes when the treatment is continuated over 1 year. Total regression of lesions due to rapamycin therapy has never been observed, except for facial angiofibromas and erythema treated with topical administration of sirolimus (Kaufman McNamara *et al.*, 2010). In fact, tumor regrowth occurred following cessation of therapy (Franz *et al.*, 2006; Wienecke *et al.*, 2006; Bissler *et al.*, 2008). These observations would implicate a life-long treatment with rapamycin, but little is known yet about long-term effects or complications of the drug. Moreover, it is unclear whether the reduction of AML size upon rapamycin treatment is associated with a reduced risk of hemorraghic complications of these tumors, the leading cause of mortality in TSC patients.

To date, no clear effect of mTORC1 inhibition on cortical tubers in TSC patients has been demonstrated. In contrast, a slightly increased number of cortical tubers in a female patient after renal transplantation has been observed after 2 years of rapamycin treatment (Tarasewicz *et al.*, 2009). This suggests that rapamycin would not be an effective drug to treat TSC-associated epilepsy and autism, which is believed to be caused by these cortical tubers. It has been suggested that these brain malformations are dysplastic rather than neoplastic, which might explain why they are not responsive to the anti-proliferative effect of rapamycin (Bissler *et al.*, 2008). However, pre-clinical studies in several mouse models showed improvement in neurological manifestations of TSC, such as seizures, and memory and learning deficits (Ehninger *et al.*, 2008; Meikle *et al.*, 2008; Zeng *et al.*, 2008). Furthermore, reduced seizure frequencies in TSC patients have been described upon treatment with rapamycin (Franz *et al.*, 2006; Muncy *et al.*, 2009). The effect of everolimus on epileptic seizure frequency in patients with TSC (aged 2 years and older) is currently investigated in a clinical trial (Table 3) (clinicaltrials.gov identifier NCT01070316).

Synopsis

Modulation of the LKB1/AMPK/TSC signaling pathway by environmental factors and genetic alterations results in aberrant mTORC1 signaling, which is observed in a wide variety of benign and malignant tumors. The distinct phenotypes associated with this aberrant signaling are difficult to interpret, however, the overall observation that mTORC1 is hyperactivated in

Table 3 Ongoing clinical trials evaluating mTORC1 inhibitors for the treatment of TSC-associated lesions

Identifier	*Patient group*	*Therapeutic agent*	*Trial design*	*Phase*	*Primary and secondary outcome measures*
NCT00789828	TSC, all ages	Everolimus	Randomized, double-blind, placebo-control, crossover, multicenter	III	Primary: SEGA size. Secondary: safety, skin lesion response, biomarkers, renal function, biomarker response and EEG changes
NCT00790400	TSC or sporadic LAM, $\geqslant$18 years	Everolimus	Randomized, double-blind, placebo-control, crossover, multicenter	III	Primary: AML size, safety. Secondary: skin lesion response, biomarkers, renal function and biomarker response
NCT00411619	TSC, $\geqslant$3 years	Everolimus	Nonrandomized, open-label, uncontrolled, single-arm, single center	I/II	Primary: adverse events, safety. Secondary: SEGA volume
NCT01031901	TSC or NF1, $\geqslant$13 years	Topical rapamycin	Randomized, double-blind, placebo-control, single center	I	Primary: safety. Secondary: skin lesion response
NCT00792766	TSC or sporadic LAM, 18–65 years	Rapamycin	Open-label, single-arm, single-centre, long-term follow-up	I/II	Primary: tolerance. Secondary: AML size
NCT01070316	TSC, $\geqslant$2 years	Everolimus	Nonrandomized, open-label, uncontrolled, single-arm, multicenter	I/II	Primary: seizure frequency. Secondary: safety
NCT00490789	TSC or sporadic LAM, 18–65 years	Rapamycin	Nonrandomized, open-label, uncontrolled, single-arm, multicenter	II	Primary: AML size, safety. Secondary: lung function
NCT00457964	TSC or sporadic LAM, 18–65 years	Everolimus	Nonrandomized, open-label, uncontrolled, single-arm, single center	I/II	Primary: AML size, safety. Secondary: other TSC-related disease including lung function in LAM
NCT00126672	TSC or sporadic LAM, 18–65 years	Rapamycin	Nonrandomized, open-label, uncontrolled, single-arm, multicenter	II	Primary: AML size, safety. Secondary: lung function, response of other TSC lesions

Abbreviations: AML, angiomyolipomas; EEG, electroencephalography; LAM, lymphangioleiomyomatosis; mTORC1, mammalian target of rapamycin complex 1; NF1, neurofibromatosis type 1; SEGA, subependymal giant cell astrocytoma; TSC, tuberous sclerosis complex.

these tumors provides hope that rationale approaches by using mTORC1 inhibitors may indeed meet clinical success. The anti-inflammatory and anti-angiogenic properties of rapamycin and its analogs are useful here, as it is well known that both inflammation and angiogenesis are linked with carcinogenesis through various mechanisms (for example, (Law, 2005; Massoumi and Sjolander, 2007)).

The success of rapamycin treatment in cancer depends on the addiction to mTORC1 activity for the tumor cells to expand. As LKB1, PTEN and NF1 are proteins a few steps upstream of the mTORC1 complex, loss of these proteins changes signaling not only leading to enhanced mTORC1 activation but also regulating mTORC1-independent signaling modules enhancing cell survival and proliferation. Therefore, these cells may be insensitive to pharmacological inhibition of mTORC1. This could be circumvented by the use of pharmacological agents affecting signaling upstream of mTORC1. For example, sorafenib, a RAF inhibitor, which inhibits the activation of ERK, is an FDA approved drug for the treatment of advanced RCC and hepatocellular carcinoma. Several PI3K/PKB inhibitors are currently being tested for clinical use in the treatment of sporadic cancers (http://www.clinicaltrial.gov). Another example is metformin, an AMPK activator, FDA approved for the treatment of diabetes mellitus type 2. Population studies have shown that metformin treatment is associated with a significant reduction of neoplasms in general, and of breast and prostate cancer in particular (Papanas *et al*., 2010). Metformin has also been suggested to serve as a candidate drug to treat PJS-associated lesions (Huang *et al*., 2008). Although the requirement for intact LKB1 to activate AMPK upon stimulation with metformin is still under debate, it has been suggested that these drugs may only be effective to treat tumors that have retained one *LKB1* allele intact (Lizcano *et al*., 2004; Shaw *et al*., 2005; Ouyang *et al*., 2010).

The TSC1:TSC2 complex is the major direct effector of mTORC1 by regulation of Rheb. However, it has previously been shown that TSC1 and TSC2 bind multiple other proteins, suggesting that the functions of the TSC1:TSC2 complex are broader (Rosner *et al*., 2008). This indicates that so far unknown effects of *TSC* mutations could affect the clinical phenotype of TSC patients. Additionally, these yet to be identified mediators involved in TSC1:TSC2 tumor suppressor function may be responsible for the differences in treatment efficiency. It should be investigated whether these yet undefined mediators are mTORC1 dependent.

Furthermore, the biological response (growth arrest versus apoptosis) of rapamycin is also known to depend on the oncogenic status of the cells, for example, rapamycin induces apoptosis in *P53*-deficient cells, whereas *P53*-proficient cells are resistant (Huang *et al*., 2003). This is particularly important for the efficacy of rapamycin treatment of sporadic cancers as it is well known that *P53* is mutated in more than half of all human sporadic cancers.

In conclusion, the impressive knowledge gained on the linear connections in signaling pathways provides targets that are useful for designing rationale therapy. However, the high levels of complexity in biological systems result in substantial unpredictability (compare for example, breast cancer (Kasper *et al*., 2009)). In this

context, the advent of systems biology techniques, such as kinome profiling, proteomics and genomics, may guide us along the long and winding road to develop adequate personalized treatments for patients with specific genetic disorders, and cancer in general.

Abbreviations

AML, angiomyolipoma; AMPK, adenosine mono-phosphate-activated protein kinase; FDA, Food and Drug Administration; LAM, lymphangioleiomyomatosis; LKB1, liver kinase B1; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NET, neuroendocrine tumor; NF1, neurofibromatosis type 1; PJS, Peutz-Jeghers syndrome; RCC, renal cell carcinoma; SEGA, subependymal giant cell astrocytoma; TSC, tuberous sclerosis complex.

Conflict of interest

The authors declare no conflict of interest.

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References

Alessi DR, Sakamoto K, Bayascas JR. (2006). LKB1-dependent signaling pathways. *Annu Rev Biochem* **75**: 137–163.

Altomare DA, Testa JR. (2005). Perturbations of the AKT signaling pathway in human cancer. *Oncogene* **24**: 7455–7464.

Baas AF, Boudeau J, Sapkota GP, Smit L, Medema R, Morrice NA *et al*. (2003). Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *EMBO J* **22**: 3062–3072.

Baybis M, Yu J, Lee A, Golden JA, Weiner H, McKhann II G *et al*. (2004). mTOR cascade activation distinguishes tubers from focal cortical dysplasia. *Ann Neurol* **56**: 478–487.

Benvenuto G, Li S, Brown SJ, Braverman R, Vass WC, Cheadle JP *et al*. (2000). The tuberous sclerosis-1 (TSC1) gene product hamartin suppresses cell growth and augments the expression of the TSC2 product tuberin by inhibiting its ubiquitination. *Oncogene* **19**: 6306–6316.

Bhaskar PT, Hay N. (2007). The two TORCs and Akt. *Dev Cell* **12**: 487–502.

Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM *et al*. (2008). Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med* **358**: 140–151.

Boudeau J, Baas AF, Deak M, Morrice NA, Kieloch A, Schutkowski M *et al*. (2003). MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. *EMBO J* **22**: 5102–5114.

Brajenovic M, Joberty G, Kuster B, Bouwmeester T, Drewes G. (2004). Comprehensive proteomic analysis of human par protein complexes reveals an interconnected protein network. *J Biol Chem* **279**: 12804–12811.

Calva D, Howe JR. (2008). Hamartomatous polyposis syndromes. *Surg Clin North Am* **88**: 779–817, vii.

Carling D, Zammit VA, Hardie DG. (1987). A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett* **223**: 217–222.

Carretero J, Medina PP, Blanco R, Smit L, Tang M, Roncador G *et al*. (2007). Dysfunctional AMPK activity, signalling through mTOR and survival in response to energetic stress in LKB1-deficient lung cancer. *Oncogene* **26**: 1616–1625.

Carsillo T, Astrinidis A, Henske EP. (2000). Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc Natl Acad Sci USA* **97**: 6085–6090.

Chan HY, Grossman AB, Bukowski RM. (2010). Everolimus in the treatment of renal cell carcinoma and neuroendocrine tumors. *Adv Ther* **27**: 495–511.

Chan JA, Zhang H, Roberts PS, Jozwiak S, Wieslawa G, Lewin-Kowalik J *et al*. (2004). Pathogenesis of tuberous sclerosis subependymal giant cell astrocytomas: biallelic inactivation of TSC1 or TSC2 leads to mTOR activation. *J Neuropathol Exp Neurol* **63**: 1236–1242.

Chong-Kopera H, Inoki K, Li Y, Zhu T, Garcia-Gonzalo FR, Rosa JL *et al*. (2006). TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. *J Biol Chem* **281**: 8313–8316.

Chung J, Kuo CJ, Crabtree GR, Blenis J. (1992). Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. *Cell* **69**: 1227–1236.

Consortium ECTS. (1993). Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* **75**: 1305–1315.

Contreras CM, Gurumurthy S, Haynie JM, Shirley LJ, Akbay EA, Wingo SN *et al*. (2008). Loss of Lkb1 provokes highly invasive endometrial adenocarcinomas. *Cancer Res* **68**: 759–766.

Cook JD, Walker CL. (2004). The Eker rat: establishing a genetic paradigm linking renal cell carcinoma and uterine leiomyoma. *Curr Mol Med* **4**: 813–824.

Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL. (2004). Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev* **18**: 1533–1538.

Corsenca A, Aebersold F, Moch H, Bird P, Weber M, Hofbauer G *et al*. (2007). Combined nephrectomy and pre-emptive renal transplantation in a tuberous sclerosis patient with angiomyolipoma, renal carcinoma and life-threatening abdominal haemorrhages. *Nephrol Dial Transplant* **22**: 3330–3333.

Curatolo P, Bombardieri R, Jozwiak S. (2008). Tuberous sclerosis. *Lancet* **372**: 657–668.

Davies DM, Johnson SR, Tattersfield AE, Kingswood JC, Cox JA, McCartney DL *et al*. (2008). Sirolimus therapy in tuberous sclerosis or sporadic lymphangioleiomyomatosis. *N Engl J Med* **358**: 200–203.

Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G. (2001). Mammalian TOR: a homeostatic ATP sensor. *Science* **294**: 1102–1105.

Dorfman J, Macara IG. (2008). STRADalpha regulates LKB1 localization by blocking access to importin-alpha, and by association with Crm1 and exportin-7. *Mol Biol Cell* **19**: 1614–1626.

Dumont FJ, Melino MR, Staruch MJ, Koprak SL, Fischer PA, Sigal NH. (1990). The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T cells. *J Immunol* **144**: 1418–1424.

Dworakowska D, Grossman AB. (2009). Are neuroendocrine tumours a feature of tuberous sclerosis? A systematic review. *Endocr Relat Cancer* **16**: 45–58.

Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ *et al*. (2008). Reversal of learning deficits in a Tsc2+/− mouse model of tuberous sclerosis. *Nat Med* **14**: 843–848.

El-Hashemite N, Walker V, Zhang H, Kwiatkowski DJ. (2003). Loss of Tsc1 or Tsc2 induces vascular endothelial growth factor production through mammalian target of rapamycin. *Cancer Res* **63**: 5173–5177.

Ess KC. (2010). Tuberous sclerosis complex: a brave new world? *Curr Opin Neurol* **23**: 189–193.

Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G *et al*. (2006). Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann Neurol* **59**: 490–498.

Godlewski J, Nowicki MO, Bronisz A, Nuovo G, Palatini J, De Lay M *et al*. (2010). MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell* **37**: 620–632.

Gurumurthy S, Hezel AF, Sahin E, Berger JH, Bosenberg MW, Bardeesy N. (2008). LKB1 deficiency sensitizes mice to carcinogen-induced tumorigenesis. *Cancer Res* **68**: 55–63.

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS *et al*. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30**: 214–226.

Habib SL, Bhandari B, Sadek N, Abboudwerner S, Abboud HE. (2010). Novel mechanism of regulation of the DNA repair enzyme OGG1 in tuberin-deficient Cells. *Carcinogenesis* **31**: 2022–2030.

Haemel AK, O'Brian AL, Teng JM. (2010). Topical rapamycin: a novel approach to facial angiofibromas in tuberous sclerosis. *Arch Dermatol* **146**: 715–718.

Haidinger M, Hecking M, Weichhart T, Poglitsch M, Enkner W, Vonbank K *et al*. (2010). Sirolimus in renal transplant recipients with tuberous sclerosis complex: clinical effectiveness and implications for innate immunity. *Transpl Int* **23**: 777–785.

Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S *et al*. (2002). Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110**: 177–189.

Hardie DG. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* **8**: 774–785.

Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP *et al*. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* **2**: 28.

Hawley SA, Selbert MA, Goldstein EG, Edelman AM, Carling D, Hardie DG. (1995). 5′-AMP activates the AMP-activated protein kinase cascade, and Ca2+/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J Biol Chem* **270**: 27186–27191.

Hay N, Sonenberg N. (2004). Upstream and downstream of mTOR. *Genes Dev* **18**: 1926–1945.

Hegedus B, Banerjee D, Yeh TH, Rothermich S, Perry A, Rubin JB *et al*. (2008). Preclinical cancer therapy in a mouse model of neurofibromatosis-1 optic glioma. *Cancer Res* **68**: 1520–1528.

Heitman J, Movva NR, Hall MN. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253**: 905–909.

Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A *et al*. (1998). A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* **391**: 184–187.

Herry I, Neukirch C, Debray MP, Mignon F, Crestani B. (2007). Dramatic effect of sirolimus on renal angiomyolipomas in a patient with tuberous sclerosis complex. *Eur J Intern Med* **18**: 76–77.

Hezel AF, Bardeesy N. (2008). LKB1; linking cell structure and tumor suppression. *Oncogene* **27**: 6908–6919.

Hezel AF, Gurumurthy S, Granot Z, Swisa A, Chu GC, Bailey G *et al*. (2008). Pancreatic LKB1 deletion leads to acinar polarity defects and cystic neoplasms. *Mol Cell Biol* **28**: 2414–2425.

Hino O. (2004). Multistep renal carcinogenesis in the Eker (Tsc 2 gene mutant) rat model. *Curr Mol Med* **4**: 807–811.

Hofbauer GF, Marcollo-Pini A, Corsenca A, Kistler AD, French LE, Wuthrich RP *et al*. (2008). The mTOR inhibitor rapamycin significantly improves facial angiofibroma lesions in a patient with tuberous sclerosis. *Br J Dermatol* **159**: 473–475.

Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y *et al*. (2009). Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* **20**: 1981–1991.

Huang J, Manning BD. (2008). The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* **412**: 179–190.

Huang S, Bjornsti MA, Houghton PJ. (2003). Rapamycins: mechanism of action and cellular resistance. *Cancer Biol Ther* **2**: 222–232.

Huang X, Wullschleger S, Shpiro N, McGuire VA, Sakamoto K, Woods YL *et al*. (2008). Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. *Biochem J* **412**: 211–221.

Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F *et al*. (2002). Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol* **22**: 7004–7014.

Ikeda Y, Sato K, Pimentel DR, Sam F, Shaw RJ, Dyck JR *et al*. (2009). Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction. *J Biol Chem* **284**: 35839–35849.

Inoki K, Ouyang H, Li Y, Guan KL. (2005). Signaling by target of rapamycin proteins in cell growth control. *Microbiol Mol Biol Rev* **69**: 79–100.

Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X *et al*. (2006). TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* **126**: 955–968.

Inoki K, Zhu T, Guan KL. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**: 577–590.

Jaleel M, McBride A, Lizcano JM, Deak M, Toth R, Morrice NA *et al*. (2005). Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate. *FEBS Lett* **579**: 1417–1423.

Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H. (2009). LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. *Physiol Rev* **89**: 777–798.

Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R *et al*. (1998). Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet* **18**: 38–43.

Jiang WG, Sampson J, Martin TA, Lee-Jones L, Watkins G, Douglas-Jones A *et al*. (2005). Tuberin and hamartin are aberrantly expressed and linked to clinical outcome in human breast cancer: the role of promoter methylation of TSC genes. *Eur J Cancer* **41**: 1628–1636.

Jishage K, Nezu J, Kawase Y, Iwata T, Watanabe M, Miyoshi A *et al*. (2002). Role of Lkb1, the causative gene of Peutz-Jegher's syndrome, in embryogenesis and polyposis. *Proc Natl Acad Sci USA* **99**: 8903–8908.

Johannessen CM, Johnson BW, Williams SM, Chan AW, Reczek EE, Lynch RC *et al*. (2008). TORC1 is essential for NF1-associated malignancies. *Curr Biol* **18**: 56–62.

Jozwiak J, Kotulska K, Lojek M, Galus R, Jozwiak S, Polnik D *et al*. (2009). Fibroblasts from normal skin of a tuberous sclerosis patient show upregulation of mTOR pathway. *Am J Dermatopathol* **31**: 68–70.

Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J *et al*. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* **20**: 1992–2003.

Kantidakis T, Ramsbottom BA, Birch JL, Dowding SN, White RJ. (2010). mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. *Proc Natl Acad Sci USA* **107**: 11823–11828.

Kasper M, Jaks V, Fiaschi M, Toftgard R. (2009). Hedgehog signalling in breast cancer. *Carcinogenesis* **30**: 903–911.

Katajisto P, Vaahtomeri K, Ekman N, Ventela E, Ristimaki A, Bardeesy N *et al*. (2008). LKB1 signaling in mesenchymal cells required for suppression of gastrointestinal polyposis. *Nat Genet* **40**: 455–459.

Kaufman McNamara E, Curtis AR, Fleischer Jr AB. (2010). Successful treatment of angiofibromata of tuberous sclerosis complex with rapamycin. *J Dermatolog Treat* (e-pub ahead of print).

Kenerson H, Dundon TA, Yeung RS. (2005). Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. *Pediatr Res* **57**: 67–75.

Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H *et al*. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110**: 163–175.

Knowles MA, Hornigold N, Pitt E. (2003). Tuberous sclerosis complex (TSC) gene involvement in sporadic tumours. *Biochem Soc Trans* **31**: 597–602.

Kobayashi T, Minowa O, Kuno J, Mitani H, Hino O, Noda T. (1999). Renal carcinogenesis, hepatic hemangiomatosis, and embryonic lethality caused by a germ-line Tsc2 mutation in mice. *Cancer Res* **59**: 1206–1211.

Koenig MK, Butler IJ, Northrup H. (2008). Regression of subependymal giant cell astrocytoma with rapamycin in tuberous sclerosis complex. *J Child Neurol* **23**: 1238–1239.

Kola B, Boscaro M, Rutter GA, Grossman AB, Korbonits M. (2006). Expanding role of AMPK in endocrinology. *Trends Endocrinol Metab* **17**: 205–215.

Krischock L, Beach R, Taylor J. (2010). Sirolimus and tuberous sclerosis-associated renal angiomyolipomas. *Arch Dis Child* **95**: 391–392.

Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P *et al*. (2010). Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* **363**: 1801–1811.

Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW. (1999). 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* **48**: 1667–1671.

Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N *et al*. (2002). A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. *Hum Mol Genet* **11**: 525–534.

Lam C, Bouffet E, Tabori U, Mabbott D, Taylor M, Bartels U. (2010). Rapamycin (sirolimus) in tuberous sclerosis associated pediatric central nervous system tumors. *Pediatr Blood Cancer* **54**: 476–479.

Law BK. (2005). Rapamycin: an anti-cancer immunosuppressant? *Crit Rev Oncol Hematol* **56**: 47–60.

Lee L, Sudentas P, Dabora SL. (2006). Combination of a rapamycin analog (CCI-779) and interferon-gamma is more effective than single agents in treating a mouse model of tuberous sclerosis complex. *Genes Chromosomes Cancer* **45**: 933–944.

Lee L, Sudentas P, Donohue B, Asrican K, Worku A, Walker V *et al*. (2005). Efficacy of a rapamycin analog (CCI-779) and IFN-gamma in tuberous sclerosis mouse models. *Genes Chromosomes Cancer* **42**: 213–227.

Lee PS, Tsang SW, Moses MA, Trayes-Gibson Z, Hsiao LL, Jensen R *et al*. (2010). Rapamycin-insensitive up-regulation of MMP2 and other genes in tuberous sclerosis complex 2-deficient lymphangioleiomyomatosis-like cells. *Am J Respir Cell Mol Biol* **42**: 227–234.

Lim CT, Kola B, Korbonits M. (2010). AMPK as a mediator of hormonal signalling. *J Mol Endocrinol* **44**: 87–97.

Lizcano JM, Goransson O, Toth R, Deak M, Morrice NA, Boudeau J *et al*. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* **23**: 833–843.

Massoumi R, Sjolander A. (2007). The role of leukotriene receptor signaling in inflammation and cancer. *ScientificWorldJournal* **7**: 1413–1421.

McCarthy A, Lord CJ, Savage K, Grigoriadis A, Smith DP, Weigelt B *et al*. (2009). Conditional deletion of the Lkb1 gene in the mouse mammary gland induces tumour formation. *J Pathol* **219**: 306–316.

McGarrity TJ, Amos C. (2006). Peutz-Jeghers syndrome: clinicopathology and molecular alterations. *Cell Mol Life Sci* **63**: 2135–2144.

Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M *et al*. (2008). Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci* **28**: 5422–5432.

Mi R, Ma J, Zhang D, Li L, Zhang H. (2009). Efficacy of combined inhibition of mTOR and ERK/MAPK pathways in treating a tuberous sclerosis complex cell model. *J Genet Genomics* **36**: 355–361.

Michels AA, Robitaille AM, Buczynski-Ruchonnet D, Hodroj W, Reina JH, Hall MN *et al*. (2010). mTORC1 directly phosphorylates and regulates human MAF1. *Mol Cell Biol* **30**: 3749–3757.

Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M *et al*. (2010). Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol* **28**: 245–255.

Miyata H, Chiang AC, Vinters HV. (2004). Insulin signaling pathways in cortical dysplasia and TSC-tubers: tissue microarray analysis. *Ann Neurol* **56**: 510–519.

Miyoshi H, Nakau M, Ishikawa TO, Seldin MF, Oshima M, Taketo MM. (2002). Gastrointestinal hamartomatous polyposis in Lkb1 heterozygous knockout mice. *Cancer Res* **62**: 2261–2266.

Moore F, Weekes J, Hardie DG. (1991). Evidence that AMP triggers phosphorylation as well as direct allosteric activation of rat liver AMP-activated protein kinase. A sensitive mechanism to protect the cell against ATP depletion. *Eur J Biochem* **199**: 691–697.

Muncy J, Butler IJ, Koenig MK. (2009). Rapamycin reduces seizure frequency in tuberous sclerosis complex. *J Child Neurol* **24**: 477.

Onda H, Crino PB, Zhang H, Murphey RD, Rastelli L, Gould Rothberg BE *et al*. (2002). Tsc2 null murine neuroepithelial cells are a model for human tuber giant cells, and show activation of an mTOR pathway. *Mol Cell Neurosci* **21**: 561–574.

Orlova KA, Crino PB. (2010). The tuberous sclerosis complex. *Ann NY Acad Sci* **1184**: 87–105.

Ouyang J, Parakhia RA, Ochs RS. (2010). Metformin activates AMP-kinase through inhibition of AMP deaminase. *J Biol Chem* **286**: 1–11.

Papanas N, Maltezos E, Mikhailidis DP. (2010). Metformin and cancer: licence to heal? *Expert Opin Investig Drugs* **19**: 913–917.

Parrinello S, Lloyd AC. (2009). Neurofibroma development in NF1–insights into tumour initiation. *Trends Cell Biol* **19**: 395–403.

Pearson HB, McCarthy A, Collins CM, Ashworth A, Clarke AR. (2008). Lkb1 deficiency causes prostate neoplasia in the mouse. *Cancer Res* **68**: 2223–2232.

Peces R, Peces C, Cuesta-Lopez E, Perez-Duenas V, Vega-Cabrera C, Azorin S *et al*. (2010). Low-dose rapamycin reduces kidney volume angiomyolipomas and prevents the loss of renal function in a patient with tuberous sclerosis complex. *Nephrol Dial Transplant* **25**: 3787–3791.

Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J *et al*. (2001). An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/− mice. *Proc Natl Acad Sci USA* **98**: 10320–10325.

Pollizzi K, Malinowska-Kolodziej I, Stumm M, Lane H, Kwiatkowski D. (2009). Equivalent benefit of mTORC1 blockade and combined PI3K-mTOR blockade in a mouse model of tuberous sclerosis. *Mol Cancer* **8**: 38.

Pressey JG, Wright JM, Geller JI, Joseph DB, Pressey CS, Kelly DR. (2010). Sirolimus therapy for fibromatosis and multifocal renal cell carcinoma in a child with tuberous sclerosis complex. *Pediatr Blood Cancer* **54**: 1035–1037.

Rennebeck G, Kleymenova EV, Anderson R, Yeung RS, Artzt K, Walker CL. (1998). Loss of function of the tuberous sclerosis 2 tumor suppressor gene results in embryonic lethality characterized by disrupted neuroepithelial growth and development. *Proc Natl Acad Sci USA* **95**: 15629–15634.

Robinson J, Lai C, Martin A, Nye E, Tomlinson I, Silver A. (2009). Oral rapamycin reduces tumour burden and vascularization in Lkb1(+/−) mice. *J Pathol* **219**: 35–40.

Rosner M, Hanneder M, Siegel N, Valli A, Hengstschlager M. (2008). The tuberous sclerosis gene products hamartin and tuberin are multifunctional proteins with a wide spectrum of interacting partners. *Mutat Res* **658**: 234–246.

Sancak O, Nellist M, Goedbloed M, Elfferich P, Wouters C, Maat-Kievit A *et al*. (2005). Mutational analysis of the TSC1 and TSC2

genes in a diagnostic setting: genotype–phenotype correlations and comparison of diagnostic DNA techniques in tuberous sclerosis complex. *Eur J Hum Genet* **13**: 731–741.
Sansal I, Sellers WR. (2004). The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* **22**: 2954–2963.
Schwartz RA, Fernandez G, Kotulska K, Jozwiak S. (2007). Tuberous sclerosis complex: advances in diagnosis, genetics, and management. *J Am Acad Dermatol* **57**: 189–202.
Sehgal SN, Baker H, Vezina C. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot (Tokyo)* **28**: 727–732.
Shackelford DB, Shaw RJ. (2009). The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer* **9**: 563–575.
Shackelford DB, Vasquez DS, Corbeil J, Wu S, Leblanc M, Wu CL *et al*. (2009). mTOR and HIF-1alpha-mediated tumor metabolism in an LKB1 mouse model of Peutz-Jeghers syndrome. *Proc Natl Acad Sci USA* **106**: 11137–11142.
Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA *et al*. (2004). The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6**: 91–99.
Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA *et al*. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* **310**: 1642–1646.
Sofer A, Lei K, Johannessen CM, Ellisen LW. (2005). Regulation of mTOR and cell growth in response to energy stress by REDD1. *Mol Cell Biol* **25**: 5834–5845.
Sparagana SP, Wilkes DC, Thompson CE, Bowers DC. (2010). Optic nerve tumor in tuberous sclerosis complex is not responsive to sirolimus. *Pediatr Neurol* **42**: 443–446.
Spicer J, Rayter S, Young N, Elliott R, Ashworth A, Smith D. (2003). Regulation of the Wnt signalling component PAR1A by the Peutz-Jeghers syndrome kinase LKB1. *Oncogene* **22**: 4752–4756.
Squarize CH, Castilho RM, Gutkind JS. (2008). Chemoprevention and treatment of experimental Cowden′s disease by mTOR inhibition with rapamycin. *Cancer Res* **68**: 7066–7072.
Tarasewicz A, Debska-Slizien A, Konopa J, Zdrojewski Z, Rutkowski B. (2009). Rapamycin as a therapy of choice after renal transplantation in a patient with tuberous sclerosis complex. *Transplant Proc* **41**: 3677–3682.
Tomlinson IP, Houlston RS. (1997). Peutz-Jeghers syndrome. *J Med Genet* **34**: 1007–1011.
Uhlmann EJ, Li W, Scheidenhelm DK, Gau CL, Tamanoi F, Gutmann DH. (2004). Loss of tuberous sclerosis complex 1 (Tsc1) expression results in increased Rheb/S6K pathway signaling important for astrocyte cell size regulation. *Glia* **47**: 180–188.
Uhlmann EJ, Wong M, Baldwin RL, Bajenaru ML, Onda H, Kwiatkowski DJ *et al*. (2002). Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. *Ann Neurol* **52**: 285–296.
van Lier MG, Wagner A, van Leerdam ME, Biermann K, Kuipers EJ, Steyerberg EW *et al*. (2010). A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory. *J Cell Mol Med* **14**: 181–197.
van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S *et al*. (1997). Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* **277**: 805–808.
van Slegtenhorst M, Nellist M, Nagelkerken B, Cheadle J, Snell R, van den Ouweland A *et al*. (1998). Interaction between hamartin and tuberin, the TSC1 and TSC2 gene products. *Hum Mol Genet* **7**: 1053–1057.
Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. (2007). Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* **9**: 316–323.
Vezina C, Kudelski A, Sehgal SN. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* **28**: 721–726.
Villanueva A, Chiang DY, Newell P, Peix J, Thung S, Alsinet C *et al*. (2008). Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* **135**: 1972–1983, 1983.e1971-1911.
Wei C, Amos CI, Zhang N, Wang X, Rashid A, Walker CL *et al*. (2008). Suppression of Peutz-Jeghers polyposis by targeting mammalian target of rapamycin signaling. *Clin Cancer Res* **14**: 1167–1171.
Wei C, Amos CI, Zhang N, Zhu J, Wang X, Frazier ML. (2009). Chemopreventive efficacy of rapamycin on Peutz-Jeghers syndrome in a mouse model. *Cancer Lett* **277**: 149–154.
Wicker LS, Boltz Jr RC, Matt V, Nichols EA, Peterson LB, Sigal NH. (1990). Suppression of B cell activation by cyclosporin A, FK506 and rapamycin. *Eur J Immunol* **20**: 2277–2283.
Wienecke R, Fackler I, Linsenmaier U, Mayer K, Licht T, Kretzler M. (2006). Antitumoral activity of rapamycin in renal angiomyolipoma associated with tuberous sclerosis complex. *Am J Kidney Dis* **48**: e27–e29.
Wienecke R, Konig A, DeClue JE. (1995). Identification of tuberin, the tuberous sclerosis-2 product. Tuberin possesses specific Rap1GAP activity. *J Biol Chem* **270**: 16409–16414.
Woodrum C, Nobil A, Dabora SL. (2010). Comparison of three rapamycin dosing schedules in A/J Tsc2+/− mice and improved survival with angiogenesis inhibitor or asparaginase treatment in mice with subcutaneous tuberous sclerosis related tumors. *J Transl Med* **8**: 14.
Xiao GH, Shoarinejad F, Jin F, Golemis EA, Yeung RS. (1997). The tuberous sclerosis 2 gene product, tuberin, functions as a Rab5 GTPase activating protein (GAP) in modulating endocytosis. *J Biol Chem* **272**: 6097–6100.
Yalon M, Ben-Sira L, Constantini S, Toren A. (2010). Regression of subependymal giant cell astrocytomas with RAD001 (Everolimus) in tuberous sclerosis complex. *Childs Nerv Syst* **27**: 179–181.
Yeung RS. (2004). Lessons from the Eker rat model: from cage to bedside. *Curr Mol Med* **4**: 799–806.
Ylikorkala A, Rossi DJ, Korsisaari N, Luukko K, Alitalo K, Henkemeyer M *et al*. (2001). Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science* **293**: 1323–1326.
Yoo LI, Chung DC, Yuan J. (2002). LKB1—a master tumour suppressor of the small intestine and beyond. *Nat Rev Cancer* **2**: 529–535.
Zeng LH, Xu L, Gutmann DH, Wong M. (2008). Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol* **63**: 444–453.
Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N *et al*. (2003a). Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. *J Clin Invest* **112**: 1223–1233.
Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. (2003b). Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol* **5**: 578–581.