

REVIEW

LKB1, the multitasking tumour suppressor kinase

P A Marignani

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Mutations in the *lkb1* gene are found in Peutz-Jeghers syndrome (PJS), with loss of heterozygosity or somatic mutations at the *lkb1* locus, suggesting the gene product, the serine/threonine kinase LKB1, may function as a tumour suppressor. Patients with PJS are at a greater risk of developing cancers of epithelial tissue origin. It is widely accepted that the presence of hamartomatous polyps in PJS does not in itself lead to the development of malignancy. The signalling mechanisms that lead to these PJS related malignancies are not well understood. However, it is evident from the recent literature that LKB1 is a multitasking kinase, with unlimited potential in orchestrating cell activity. Thus far, LKB1 has been found to play a role in chromatin remodelling, cell cycle arrest, Wnt signalling, cell polarity, and energy metabolism, all of which may require the tumour suppressor function of this kinase and/or its catalytic activity.

gene,^{17–20–22} with a smaller proportion of individuals presenting with sporadic PJS,^{20–23} and a single family presenting with complete germline deletion of the *lkb1* gene.²⁴ The heterogeneity of PJS suggests the possibility of the involvement of other loci, working alone or in concert with the *lkb1* gene, a scenario that cannot be ruled out.

Inactivating mutations in *lkb1* have also been found in patients without PJS, such as those with sporadic lung adenocarcinoma, where as many as 33% of the lesions analysed displayed somatic mutations in the *lkb1* gene^{16–25–28}; ovarian carcinoma¹⁴; breast cancer¹³; and pancreatic and biliary adenocarcinoma.²⁹ The likelihood that other cancers harbour mutations in the *lkb1* gene is very possible, so that in the future there will probably be a greater number of reported mutations in the *lkb1* gene, possibly similar in scale to those reported for TP53 and PTEN.

PJS is unlike other polyposis syndromes in that the inactivation of *lkb1* occurs in epithelial cells,³⁰ whereas in juvenile polyposis syndrome (JPS), the inactivation of the *smad4* (SMA and MAD related protein 4) gene occurs in stromal cells.³¹ In both syndromes, the loss of *lkb1* and *smad4*, respectively, leads to the formation of benign hamartomatous polyps composed primarily of stromal tissue. In contrast, malignant tumours derived from PJS and JPS polyps are comprised primarily of epithelial cells.³¹ Understanding the molecular mechanisms that mediate PJS early stage benign stromal lesions and PJS related later stage epithelial malignancy is extremely important, and is probably relevant to the development of future targeted treatments directed towards later stage malignancies.

LKB1, THE KINASE

Most of the identified mutations in the *lkb1* gene are localised to the catalytic (kinase) domain of LKB1, so that it is thought that PJS results from a loss of LKB1 kinase activity.^{20–32–33} Therefore, the discovery that mutations in the tumour suppressor *lkb1* gene are responsible for a hamartomatous polyposis syndrome is unique because, to date, PJS is the only cancer susceptibility syndrome that has been shown to result from loss of the catalytic activity of a serine/threonine kinase.^{8–17} It is assumed that the

Peutz-Jeghers syndrome (PJS) was first identified by a Dutch physician Peutz in 1921,¹ and later by an American physician Jeghers in 1949.² PJS is an autosomal dominant disorder, characterised by mucocutaneous hyperpigmentation and multiple benign gastrointestinal hamartomatous polyps.^{3–4} The relative incidence of PJS is approximately 1/120 000 births.⁵ Patients with PJS almost always develop malignancies of the epithelial tissues, particularly of the gastrointestinal tract. For example, they have an 84 fold increased risk of developing colon cancer, a 213 fold increased risk of gastric cancers, and a 520 fold increased risk of developing small intestinal cancers.^{6–7} Additional PJS related malignancies include cancers of the breast, lung, uterus, ovaries, cervix, and testes.^{8–16} The molecular mechanisms that underlie these malignancies are not fully understood.

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The gene responsible for PJS has been identified by linkage analysis on chromosome 19p13.3 and encodes a novel serine/threonine kinase, LKB1 (STK11).^{17–19} Most (60–70%) patients with PJS show germline mutations in the *lkb1*

Correspondence to:
Dr P A Marignani,
Dalhousie University,
Faculty of Medicine,
Department of
Biochemistry and
Molecular Biology, Sir
Charles Tupper Medical
Building, Room 9S1, 5850
College Street, Halifax,
Nova Scotia B3H-1X5,
Canada; pmarigna@
dal.ca

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Abbreviations: AMPK, 5'-AMP activated protein kinase; Brg1, Brahma related gene 1; Dvl, Dishevelled; JPS, juvenile polyposis syndrome; GSK-3 β , glycogen synthase kinase 3 β ; LIP1, LKB1 interacting protein; par, partitioning-defective gene; PJS, Peutz-Jeghers syndrome; PKC, protein kinase C; *smad4*, SMA and MAD related protein 4; STRAD, STE20 related adaptor; VEGF, vascular endothelial growth factor; XEEK1, *Xenopus laevis* egg and embryonic kinase 1

tumour suppressor function of LKB1 is to trans-phosphorylate protein targets that are relevant to tumour progression.

LKB1 is a 433 residue serine threonine protein kinase that, until recently, was categorised as a member of the AGC superfamily of kinases (referring to multiple related kinase families having a highly conserved kinase domain; PKA, PKG, PKC). The classification of serine threonine kinases is rapidly changing as additional functional information on each of the kinases is acquired. Because of this, LKB1 has now been classified as a member of the calcium/calmodulin regulated kinase-like family that is part of the Ca²⁺/calmodulin kinase group of kinases (<http://www.kinase.com>). LKB1 orthologues include *Xenopus laevis* egg and embryonic kinase 1 (XEEK1),³⁴ mouse LKB1,³⁵ *Caenorhabditis elegans* partitioning defective gene 4 (par-4),³⁶ and drosophila LKB1.³⁷ Par-4 and drosophila LKB1 share 26% and 44% overall identity with human LKB1, respectively, and 42% and 66% identity with the LKB1 kinase domain, respectively. Human LKB1, mouse LKB1, and XEEK1 share a conserved nuclear localisation signal, and LKB1 localises both in the cytoplasm and the cell nucleus.³⁸ However, the intracellular distribution is dependent on its interaction with binding partners, such as the exclusively nuclear chromatin remodelling protein, Brahma related gene 1 (Brg1),³⁹ the cytoplasmic LKB1 interacting protein (LIP1),⁴⁰ STE20 related adaptor (STRAD), and scaffolding protein MO25.^{41–43} LKB1 has also been seen in the mitochondria,⁷ and at the cellular membrane, through a conserved CAAX box.^{44–45} The introduction of LKB1 into human G361 melanoma cells that are defective in lkb1 expression leads to growth suppression and G1 cell cycle arrest, whereas the introduction of kinase defective forms of LKB1 has no such effect.^{32–33–45–46} These findings support a role for LKB1 as a tumour suppressor. LKB1 is regulated by a series of upstream kinases, specifically, LKB1 is phosphorylated on serine 431 (S431) by cAMP dependent protein kinase A or by p90 ribosomal S6 kinase.⁴⁵ LKB1 is also phosphorylated on S31, S325, threonine 336 (T336), and T366 in vitro, but it is not known which kinases mediate these phosphorylation events.⁴⁷ LKB1 may be involved in the tumour suppressor p53 signalling pathways and apoptosis because LKB1 phosphorylates recombinant p53 in vitro and is involved in the expression of p53 responsive genes.^{7–26–47}

LKB1 DISRUPTION IN ANIMAL MODELS

LKB1 has a role in early mammalian embryonic development, as does its *X laevis* orthologue, XEEK1.³⁴ The inhibition of XEEK1 expression in xenopus embryos results in developmental abnormalities reminiscent of Wnt signalling defects.⁴⁸ Lkb1^{-/-} mice die at midgestation and display abnormal neural tube development, mesenchymal cell death, defective somitogenesis, and abnormal vasculature associated with raised levels of vascular endothelial growth factor (VEGF), possibly through the deregulation of VEGF mediated signalling.⁴⁹ These findings are similar to situations where the loss of von Hippel-Lindau protein leads to deregulation of VEGF signalling.⁴⁹ In contrast, lkb1^{+/-} mice develop hamartomatous tumours with similar histopathology to those found in patients with PJS, although the absolute location of the tumours within the mouse intestine differs from that seen in humans.⁵⁰ Interestingly, this same study found that these tumours were not caused by biallelic inactivation of lkb1 because the mice retained a wild-type copy of lkb1, but rather were the result of haploinsufficiency.⁵⁰ Similar observations regarding haploinsufficiency have been described for other tumour suppressors such as p27^{kip}—tumours develop in p27^{kip+/-} mice despite the presence of a wild-type copy of p27^{kip}.⁵¹ Another member of the hamartomatous polyposis syndrome family, JPS, has been attributed, in part, to loss of

heterozygosity at the smad4 locus in humans and in mice. However, in some instances smad4^{+/-} mice develop cancers despite the retention of a wild-type copy of smad4.⁵² Haploinsufficiency may be one explanation for the development of polyps and cancers in PJS and JPS.^{50–52–53} However a recent mouse study reported quite the opposite, namely the loss of the wild-type lkb1 copy in a subset of lkb1^{+/-} polyps.⁵⁴ In this same study, the authors propose that the loss of lkb1 in healthy epithelial intestinal tissues is protective, particularly if the loss is an early event, whereas the loss of lkb1 at a later stage, such as in cells that have already undergone malignant transformation as a result of other cancer genes, facilitates cancer progression.⁵⁴ Clearly, findings from lkb1 knockout studies leave numerous questions as to the genetic mechanism involved in both polyposis formation and the steps that lead to malignant progression in PJS.

LKB1 SIGNALLING

Over the past seven years, LKB1 has shown an aptitude for multitasking. When one considers that the first LKB1 binding partner Brg1 was identified only four years ago,³⁹ and the more recent evidence that LKB1 is involved in Wnt signalling,^{48–55} in cell polarity,^{37–56} and in energy metabolism,^{57–58} the biological networking capability of LKB1 is palpable. There are probably additional pathways, yet to be described, in which LKB1 is involved through protein–protein interactions and/or through trans-phosphorylation events. On a cautionary note, it is arguable whether the multiplexing of LKB1 networks is a realistic representation of cellular signalling events in vivo. The question is whether these signalling networks play a part in the tumour suppressor function of LKB1 and whether they are suitable targets for the development of specific treatments.

LKB1 IN CHROMATIN REMODELLING

LKB1 is known to associate with the ATPase Brg1 in vivo,³⁹ an essential component of the human SWI/SNF chromatin remodelling complex. In eukaryotes, the basic subunit of chromatin is the nucleosome. A function of nucleosomes is to regulate gene transcription by mediating the compaction of DNA. However, transient disruptions in nucleosomes allow protein–DNA interactions to take place by using the energy derived from Brg1 ATPase mediated ATP hydrolysis to disrupt nucleosome structure, allowing the helicase to unwind double stranded DNA.^{59–63} In the presence of LKB1, the ATPase activity of Brg1 is enhanced.³⁹ Because LKB1 induces G1 growth arrest³² and associates with Brg1,³⁹ which is involved in retinoblastoma protein induced cell cycle arrest in both the G1 and S phases,^{32–64} LKB1 may function in the Brg1 signalling pathway to induce growth arrest.³⁹ The introduction of Brg1 into SW13 cells that lack Brg1 expression leads to the appearance of large flat cells, indicative of cells that have undergone growth arrest and are senescent.^{64–65}

“LKB1 induces G1 growth arrest and associates with Brg1, which is involved in retinoblastoma protein induced cell cycle arrest in both the G1 and S phases”

The coexpression of inactive LKB1 kinase, SL26, and Brg1 results in a significant reduction in the number of senescent SW13 cells compared with expression of Brg1 alone and with the coexpression of Brg1 together with LKB1.³⁹ Because the allelic mutant SL26 lacks protein kinase activity, but binds to and stimulates Brg1 ATPase activity, these findings indicate that LKB1 protein kinase activity is required for Brg1 mediated growth arrest, but is not required for Brg1–ATPase activity.³⁹

Other interacting partners of LKB1 include LIP,⁴⁰ an anchoring protein that tethers LKB1 to the cytoplasmic membrane and binds to transforming growth factor β regulated transcription factor Smad4, forming an LKB1–LIP–Smad4 ternary complex. LIP functions to regulate the distribution of LKB1 between the cytoplasm and nucleus, where it can associate with interacting partners and/or phosphorylate substrates. LKB1 has also been implicated in Wnt signalling, with two opposing observations. In the first study, XEEK1 was found to associate with and regulate the phosphorylation of glycogen synthase kinase 3 β (GSK-3 β), in addition to associating with a known GSK-3 β kinase, protein kinase C- ζ (PKC- ζ). The authors provide compelling evidence in vivo that XEEK1/LKB1 enhances Wnt mediated signalling.⁴⁸ In contrast, others have found that LKB1 is an upstream kinase of the partitioning defective serine threonine kinase, Par-1A, regulating its phosphorylation and activation.⁵⁵ Specifically, LKB1 was found to compete with Dishevelled (Dvl; a protein involved in Wnt mediated signalling) for Par-1A. By redirecting Dvl from interacting with Par-1A, LKB1 suppresses Dvl mediated Wnt mediated signalling.⁵⁵

LKB1 INVOLVEMENT IN CELL POLARITY

The localisation and kinase activity of LKB1 is regulated by two recently discovered proteins, STRAD and MO25.^{41–42} The STRAD proteins, STRAD α and STRAD β , are non-functional kinases because they lack residues within the kinase domain that are essential for their catalytic activity. The STE20-like kinases were first identified in yeast,⁶⁶ and are most similar to mammalian mitogen activated kinases. When in complex with STRAD pseudokinases and the stabilising proteins MO25 α/β , LKB1 is relocated from the nucleus to the cytoplasm.^{41–42}

The spatial and temporal movement of cells to their biologically relevant location during eukaryotic development is crucial for the survival of the organism. The genetic control of cellular polarisation is mediated by signalling pathways that are conserved from invertebrates to vertebrates. It is widely accepted that the loss of cell polarity is a contributing factor in the epithelial–mesenchymal transition that arises during cellular transformation.⁶⁷ The *C. elegans* par genes, par1–6, were identified as maternal effect mutations that caused disproportional partitioning of polar granules at the one cell stage during embryonic asymmetric division.^{68–69} A decade after this discovery, research groups determined that a complex of three proteins was required to establish anterior–posterior polarity at the one cell stage asymmetric division in *C. elegans*—Par-3, Par-6, and atypical PKC- λ and PKC- ζ .⁷⁰ More recently, LKB1, the putative Par-4 homologue, has been found to provoke polarity in single isolated cells in a STRAD inducible system.⁵⁶ In PJS, the loss of lkb1 gene expression leads to depolarisation of intestinal cells, which in turn leads to cell transformation and the malignancies associated with the disease. For recent reviews see Boudeau *et al.*^{42–71}

LKB1 IN CELL METABOLISM

LKB1 has been implicated in metabolism and cell proliferation through its regulation of the metabolic stress kinase family, 5'-AMP activated protein kinase (AMPK).^{43–57} During metabolic stress, the ratio of cellular AMP to ATP is increased, AMPK senses the change in ATP values, and is activated to restore the energy integrity of the cell.⁷² The yeast orthologue of AMPK is Snf1, and it has three known upstream kinases, namely: Elm1, Pak1, and Tos3.^{73–74} In the mammalian system, LKB1 shows sequence similarity to Elm1, Pak1, and Tos3, and functions as an upstream kinase of AMPK, in essence an AMPK kinase.⁵⁷ More recently, when

Take home messages

- Mutations in the LKB1 gene are found in Peutz-Jeghers syndrome (PJS), in which there is a greater risk of developing cancers of epithelial tissue origin
- This suggests that the gene product, the serine/threonine kinase LKB1, may function as a tumour suppressor, particularly because most mutations are located in the catalytic domain
- The signalling mechanisms that lead to PJS related malignancies are still being dissected, but recent evidence suggests that LKB1 is a multitasking kinase, with unlimited potential in orchestrating cell activity
- To date, LKB1 has been found to play a role in chromatin remodelling, cell cycle arrest, Wnt signalling, cell polarity, and energy metabolism, all of which may require the tumour suppressor function of this kinase and/or its catalytic activity
- As more LKB1 signalling pathways are identified, a more profound understanding of mechanisms that lead to PJS and associated malignancies will give rise to the development of targeted cancer treatments

in complex with STRAD and MO25, LKB1 has been shown to regulate 11 of the 12 AMPK family members in vitro, including MARK/PAR-1,^{43–58} suggesting that one of the tumour suppressor functions of LKB1 may be the regulation of AMPK signalling. For more details on the role of LKB1 in metabolism see the reviews by Baas *et al.*, Boudeau *et al.* and Kynakis.^{71–75–76}

“LKB1 has been implicated in metabolism and cell proliferation through its regulation of the metabolic stress kinase family, 5'-AMP activated protein kinase”

A major challenge for scientists intent on fully understanding the function of LKB1 in disease will be first to unravel the normal signalling pathway(s) mediated by this kinase in vivo—for example, by identifying the true substrates, assuming that the primary function of LKB1 is to invoke trans-phosphorylation events as part of its tumour suppressor function, and by continuing to identify the interacting partners. As additional LKB1 signalling pathways are identified, a more profound understanding of mechanisms that lead to PJS and associated malignancies will give rise to the development of targeted cancer treatments. There is still a great deal to learn about LKB1; one need only to reflect on the ongoing saga of the most widely studied and disputed tumour suppressor, p53, to realise the complexity of this task.

REFERENCES

- 1 Peutz J. A very remarkable case of familial polyposis of mucous membrane of intestinal tract and accompanied by peculiar pigmentations of skin and mucous membrane [Dutch]. *Nederlands Tijdschrift voor Geneeskunde* 1921;10:134–46.
- 2 Jeghers H, McKusick V, Katz K. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. *N Engl J Med* 1949;241:992–1005.
- 3 Hemminki A. The molecular basis and clinical aspects of Peutz-Jeghers syndrome. *Cell Mol Life Sci* 1999;55:735–50.
- 4 Wirtzfeld DA, Petrelli NJ, Rodriguez-Bigas MA. Hamartomatous polyposis syndromes: molecular genetics, neoplastic risk, and surveillance recommendations. *Ann Surg Oncol* 2001;8:319–27.
- 5 Lindor NM, Greene MH. The concise handbook of family cancer syndromes. Mayo familial cancer program. *J Natl Cancer Inst* 1998;90:1039–71.
- 6 Giardiello FM, Welsh SB, Hamilton SR, *et al.* Increased risk of cancer in the Peutz-Jeghers syndrome. *N Engl J Med* 1987;316:1511–14.

- 7 **Karuman P**, Gozani O, Odze RD, *et al*. The Peutz-Jegher gene product LKB1 is a mediator of p53-dependent cell death. *Mol Cell* 2001;**7**:1307–19.
- 8 **Avizienyte E**, Roth S, Loukola A, *et al*. Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors. *Cancer Res* 1998;**58**:2087–90.
- 9 **Boardman LA**, Couch FJ, Burgart LJ, *et al*. Genetic heterogeneity in Peutz-Jeghers syndrome [in process citation]. *Hum Mutat* 2000;**16**:23–30.
- 10 **Cannolly DC**, Katabuchi H, Cliby WA, *et al*. Somatic mutations in the STK11/LKB1 gene are uncommon in rare gynecological tumor types associated with Peutz-Jegher's syndrome. *Am J Pathol* 2000;**156**:339–45.
- 11 **Hruban RH**, Petersen GM, Goggins M, *et al*. Familial pancreatic cancer. *Ann Oncol* 1999;**10**(suppl 4):69–73.
- 12 **Nishioka Y**, Kobayashi K, Sagae S, *et al*. Mutational analysis of STK11 gene in ovarian carcinomas. *Jpn J Cancer Res* 1999;**90**:629–32.
- 13 **Shen Z**, Wen XF, Lan F, *et al*. The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma. *Clin Cancer Res* 2002;**8**:2085–90.
- 14 **Papageorgiou T**, Stratakis KA. Ovarian tumors associated with multiple endocrine neoplasias and related syndromes (Carney complex, Peutz-Jeghers syndrome, von Hippel-Lindau disease, Cowden's disease). *Int J Gynecol Cancer* 2002;**12**:337–47.
- 15 **Sato N**, Rosty C, Jansen M, *et al*. STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. *Am J Pathol* 2001;**159**:2017–22.
- 16 **Sanchez-Cespedes M**, Parrella P, Esteller M, *et al*. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. *Cancer Res* 2002;**62**:3659–62.
- 17 **Hemminki A**, Tomlinson I, Markie D, *et al*. Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis [see comments]. *Nat Genet* 1997;**15**:87–90.
- 18 **Hemminki A**, Markie D, Tomlinson I, *et al*. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* 1998;**391**:184–7.
- 19 **Jenne DE**, Reimann H, Nezu J, *et al*. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet* 1998;**18**:38–43.
- 20 **Ylikorkala A**, Avizienyte E, Tomlinson IP, *et al*. Mutations and impaired function of LKB1 in familial and non-familial Peutz-Jeghers syndrome and a sporadic testicular cancer. *Hum Mol Genet* 1999;**8**:45–51.
- 21 **Olshwang S**, Boisson C, Thomas G. Peutz-Jeghers families unlinked to STK11/LKB1 gene mutations are highly predisposed to primitive biliary adenocarcinoma. *J Med Genet* 2001;**38**:356–60.
- 22 **Lim W**, Hearle N, Shah B, *et al*. Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. *Br J Cancer* 2003;**89**:308–13.
- 23 **Scott RJ**, Crooks R, Meldrum CJ, *et al*. Mutation analysis of the STK11/LKB1 gene and clinical characteristics of an Australian series of Peutz-Jeghers syndrome patients. *Clin Genet* 2002;**62**:282–7.
- 24 **Le Meur N**, Martin C, Saugier-Verber P, *et al*. Complete germline deletion of the STK11 gene in a family with Peutz-Jeghers syndrome. *Eur J Hum Genet* 2004;**12**:415–18.
- 25 **Carretero J**, Medina PP, Pio R, *et al*. Novel and natural knockout lung cancer cell lines for the LKB1/STK11 tumor suppressor gene. *Oncogene* 2004;**23**:4037–40.
- 26 **Jimenez AI**, Fernandez P, Dominguez O, *et al*. Growth and molecular profile of lung cancer cells expressing ectopic LKB1: down-regulation of the phosphatidylinositol 3'-phosphate kinase/PTEN pathway. *Cancer Res* 2003;**63**:1382–8.
- 27 **Ghaffar H**, Sahin F, Sanchez-Cepedes M, *et al*. LKB1 protein expression in the evolution of glandular neoplasia of the lung. *Clin Cancer Res* 2003;**9**:2998–3003.
- 28 **Fernandez P**, Carretero J, Medina PP, *et al*. Distinctive gene expression of human lung adenocarcinomas carrying LKB1 mutations. *Oncogene* 2004;**33**:5084–91.
- 29 **Sahin F**, Maitra A, Argani P, *et al*. Loss of Stk11/Lkb1 expression in pancreatic and biliary neoplasms. *Mod Pathol* 2003;**16**:686–91.
- 30 **Wang ZJ**, Ellis I, Zauber P, *et al*. Allelic imbalance at the LKB1 (STK11) locus in tumours from patients with Peutz-Jeghers' syndrome provides evidence for a hamartoma-(adenoma)-carcinoma sequence [see comments]. *J Pathol* 1999;**188**:9–13.
- 31 **van der Weyden L**, Jonkers J, Bradley A. Cancer: stuck at first base. *Nature* 2002;**419**:127–8.
- 32 **Tainen M**, Ylikorkala A, Makela TP. Growth suppression by Lkb1 is mediated by a G(1) cell cycle arrest. *Proc Natl Acad Sci U S A* 1999;**96**:9248–51.
- 33 **Boudeau J**, Kieloch A, Alessi DR, *et al*. Functional analysis of LKB1/STK11 mutants and two aberrant isoforms found in Peutz-Jeghers syndrome patients. *Hum Mutat* 2003;**21**:172.
- 34 **Su JY**, Erikson E, Maller JL. Cloning and characterization of a novel serine/threonine protein kinase expressed in early *Xenopus* embryos. *J Biol Chem* 1996;**271**:14430–7.
- 35 **Smith DP**, Spicer J, Smith A, *et al*. The mouse Peutz-Jeghers syndrome gene Lkb1 encodes a nuclear protein kinase. *Hum Mol Genet* 1999;**8**:1479–85.
- 36 **Watts JL**, Morton DG, Bestman J, *et al*. The *C. elegans* par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. *Development* 2000;**127**:1467–75.
- 37 **Martin SG**, St Johnston D. A role for *Drosophila* LKB1 in anterior-posterior axis formation and epithelial polarity. *Nature* 2003;**421**:379–84.
- 38 **Nezu J**, Oku A, Shimane M. Loss of cytoplasmic retention ability of mutant LKB1 found in Peutz-Jeghers syndrome patients. *Biochem Biophys Res Commun* 1999;**261**:750–5.
- 39 **Marignani PA**, Kanai F, Carpenter CL. LKB1 associates with Brg1 and is necessary for Brg1-induced growth arrest. *J Biol Chem* 2001;**276**:32415–18.
- 40 **Smith DP**, Rayter SI, Niederlander C, *et al*. LIP1, a cytoplasmic protein functionally linked to the Peutz-Jeghers syndrome kinase LKB1. *Hum Mol Genet* 2001;**10**:2869–77.
- 41 **Baas AF**, Boudeau J, Sapkota GP, *et al*. Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *EMBO J* 2003;**22**:3062–72.
- 42 **Boudeau J**, Baas AF, Deak M, *et al*. MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. *EMBO J* 2003;**22**:5102–14.
- 43 **Hawley SA**, Boudeau J, Reid JL, *et al*. Complexes between the LKB1 tumor suppressor, STRADalpha/beta and MO25alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2003;**2**:28, Epub 2003 Sept 28.
- 44 **Collins SP**, Reoma JL, Gamm DM, *et al*. LKB1, a novel serine/threonine protein kinase and potential tumour suppressor, is phosphorylated by cAMP-dependent protein kinase (PKA) and prenylated in vivo. *Biochem J* 2000;**345**:673–80.
- 45 **Sapkota GP**, Kieloch A, Lizzano JM, *et al*. Phosphorylation of the protein kinase mutated in Peutz-Jeghers cancer syndrome, LKB1/STK11, at Ser431 by p90RSK and PKA but not its farnesylation at Cys433 are essential for LKB1 to suppress cell growth. *J Biol Chem* 2001;**276**:19469–82.
- 46 **Tainen M**, Vaachomeri K, Ylikorkala A, *et al*. Growth arrest by the LKB1 tumor suppressor: induction of p21(WAF1/CIP1). *Hum Mol Genet* 2002;**11**:1497–504.
- 47 **Sapkota GP**, Boudeau J, Deak M, *et al*. Identification and characterization of four novel phosphorylation sites (Ser31, Ser325, Thr336 and Thr366) on LKB1/STK11, the protein kinase mutated in Peutz-Jeghers cancer syndrome. *Biochem J* 2002;**362**:481–90.
- 48 **Ossipova O**, Bardeesy N, DePinho RA, *et al*. LKB1 (XEEK1) regulates Wnt signalling in vertebrate development. *Nat Cell Biol* 2003;**14**:14.
- 49 **Ylikorkala A**, Rossi DJ, Korsisaari N, *et al*. Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science* 2001;**293**:1323–6.
- 50 **Miyoshi H**, Nakau M, Ishikawa TO, *et al*. Gastrointestinal hamartomatous polyposis in Lkb1 heterozygous knockout mice. *Cancer Res* 2002;**62**:2261–6.
- 51 **Fero ML**, Randel E, Gurley KE, *et al*. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature* 1998;**396**:177–80.
- 52 **Xu X**, Brodie SG, Yang X, *et al*. Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. *Oncogene* 2000;**19**:1868–74.
- 53 **Jishage K**, Nezu J, Kawase Y, *et al*. Role of Lkb1, the causative gene of Peutz-Jegher's syndrome, in embryogenesis and polyposis. *Proc Natl Acad Sci U S A* 2002;**99**:8903–8.
- 54 **Bardeesy N**, Sinha M, Hezel AF, *et al*. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature* 2002;**419**:162–7.
- 55 **Spicer J**, Rayter S, Young N, *et al*. Regulation of the Wnt signalling component PARI1A by the Peutz-Jeghers syndrome kinase LKB1. *Oncogene* 2003;**22**:4752–6.
- 56 **Baas AF**, Kuipers J, van der Wel NN, *et al*. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* 2004;**116**:457–66.
- 57 **Woods A**, Johnstone SR, Dickerson K, *et al*. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 2003;**13**:2004–8.
- 58 **Lizzano JM**, Goransson O, Toth R, *et al*. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* 2004;**23**:833–43.
- 59 **Kwon H**, Imbalzano AN, Khavari PA, *et al*. Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex [see comments]. *Nature* 1994;**370**:477–81.
- 60 **Wang W**, Cote J, Xue Y, *et al*. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *EMBO J* 1996;**15**:5370–82.
- 61 **Muchardt C**, Reyes JC, Bourachot B, *et al*. The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J* 1996;**15**:3394–402.
- 62 **Sif S**, Stukenberg PT, Kirschner MW, *et al*. Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. *Genes Dev* 1998;**12**:2842–51.
- 63 **Phelan ML**, Sif S, Narlikar GJ, *et al*. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol Cell* 1999;**3**:247–53.
- 64 **Strobeck MW**, Knudsen KE, Fribourg AF, *et al*. BRG-1 is required for RB-mediated cell cycle arrest. *Proc Natl Acad Sci U S A* 2000;**97**:7748–53.
- 65 **Shanahan F**, Seghezzi W, Parry D, *et al*. Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters the ability of BRG1 to induce growth arrest. *Mol Cell Biol* 1999;**19**:1460–9.
- 66 **Leberer E**, Dignard D, Harcus D, *et al*. The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein beta gamma subunits to downstream signalling components. *EMBO J* 1992;**11**:4815–24.
- 67 **Thiery JP**. Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 2003;**15**:740–6.
- 68 **Kemphues KJ**, Kusch M, Wolf N. Maternal-effect lethal mutations on linkage group II of *Caenorhabditis elegans*. *Genetics* 1988;**120**:977–86.
- 69 **Brajenovic M**, Joberty G, Kuster B, *et al*. Comprehensive proteomic analysis of human Par protein complexes reveals an interconnected protein network. *J Biol Chem* 2004;**279**:12804–11.
- 70 **Izumi Y**, Hirose T, Tamai Y, *et al*. An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J Cell Biol* 1998;**143**:95–106.
- 71 **Baas AF**, Smit L, Clevers H. LKB1 tumor suppressor protein: PARtaker in cell polarity. *Trends Cell Biol* 2004;**14**:312–19.
- 72 **Hardie DG**, Salt IP, Hawley SA, *et al*. AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. *Biochem J* 1999;**338**:717–22.

- 73 **Hong SP**, Leiper FC, Woods A, *et al.* Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc Natl Acad Sci U S A* 2003;**7**:7.
- 74 **Sutherland CM**, Hawley SA, McCartney RR, *et al.* Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. *Curr Biol* 2003;**13**:1299–305.
- 75 **Boudeau J**, Sapkota G, Alessi DR. LKB1, a protein kinase regulating cell proliferation and polarity. *FEBS Lett* 2003;**546**: 159–65.
- 76 **Kyriakis JM**. At the crossroads: AMP-activated kinase and the LKB1 tumor suppressor link cell proliferation to metabolic regulation. *J Biol* 2003;**2**:26. Epub 2003 Oct 22.

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