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Lkb1 deletion in murine B lymphocytes promotes cell death and cancer

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Abstract

LKB1 (aka *STK11*) is a potent tumor suppressor in solid tumors such as melanoma and lung adenocarcinoma, but inactivation in hematopoietic cells causes cell death, without signs of tumorigenesis. We noted somatic *LKB1* deletion or mutation at low frequency in human B cell lymphoma. In order to determine if *LKB1* inactivation is a passenger or driver event in lymphoid cancers, we examined the effects of conditional inactivation of *Lkb1* in murine lymphocytes. Consistent with prior reports, *Lkb1* deletion in either T or B cells resulted in massive, lineage-specific apoptosis. Surprisingly, however, despite an 80% reduction of peripheral B cell number, animals harboring somatic B-lineage *Lkb1* deletion developed aggressive B cell lymphoma with high penetrance and moderate latency. Malignant B cells demonstrated somatic *Lkb1* recombination. In contrast, *Lkb1* deletion in T cells did not promote tumorigenesis. Concomitant Ras activation with *Lkb1* deletion reduced T cell apoptosis but did not enhance tumor formation in T or B cells. These results suggest that while physiologic *LKB1* expression exerts a potent pro-survival effect in lymphocytes, *LKB1* inactivation nonetheless facilitates transformation of B, but not T, lymphocytes.

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AUTHOR CONTRIBUTIONS

G.P.S and N.E.S conceived the study, designed experiments and wrote the manuscript. G.P.S performed experiments. Y.F. performed animal histopathology. G.P.S, Y.F, L.M.S and N.E.S analyzed and interpreted the data.

INTRODUCTION

Hypomorphic mutations of the liver kinase B1 gene (*LKB1*), also known as serine threonine kinase 11 (*STK11*), cause Peutz-Jeghers syndrome (PJS) (1), which is characterized by gastrointestinal hamartomas and mucocutaneous hyperpigmentation. *LKB1* is a serine/threonine kinase that activates a large family of downstream kinases, thereby influencing myriad cancer-relevant pathways of cell metabolism, survival, polarity, invasion and migration (2, 3). *LKB1* is considered an important tumor suppressor gene in solid tumors. PJS patients exhibit a moderate predisposition to solid malignancies, including gastroesophageal, colorectal, pancreatic and breast cancers (4), with age-cumulative risks also including lung cancers (5). Somatic mutations in *LKB1* have been observed with high frequency in lung adenocarcinoma (6–8), cholangiocarcinoma (7), cervical carcinoma (9) and melanoma (10–12). Correspondingly, studies featuring somatic deletion of *Lkb1* in specific tissues have shown that its inactivation promotes tumorigenesis and metastatic spread in a variety of solid tumor models (e.g. lung, pancreas, cervix, uterus and melanoma), frequently demonstrating potent cooperation with RAS-pathway activation.

The role of *LKB1* in hematopoietic tissues, however, is less clear. PJS patients do not appear to exhibit an excess of lymphoma or leukemia. Moreover, inactivation of *Lkb1* in murine hematopoietic stem cells (HSCs) (13–15) and T cells (16) results in near complete depletion of these compartment due to apoptosis, with no apparent increase in transformation. These findings suggest that physiological *LKB1* expression exerts a potent pro-survival effect in hematopoietic cells, which would be more consistent with an oncogenic, rather than tumor suppressive, role in such tissues.

The above reasoning was called into question, however, by multiple array comparative genomic hybridization (array-CGH) studies that identified 19p13 chromosomal deletions or translocations in B cell malignancies (17–21). Although 19p13 deletions in these studies were not very common in B cell lymphomas, a recent study reported that 19p13.3 deletions occur in as high as 8% of diffuse large B cell lymphomas (DLBCL) (22). As *LKB1* is located on chromosome 19p with a number of other known tumor suppressor genes (e.g. *BRG1/SMARCA4*, *SIRT6*, *TNFSF7*, *TNFSF9*), it still remains unclear whether other genes within this region possess tumor-suppressive properties and contribute towards lymphoma development. Given the well-established function of *LKB1* in solid tumors as a tumor-suppressor, we considered the possibility that *LKB1* inactivation in these tumors contributed towards the development of cancer. Toward that end, we studied the ability of somatic inactivation of *Lkb1* in murine T or B cells, alone and on the context of concomitant Ras-pathway activation.

RESULTS

Even though 19p13 deletions have been previously identified in lymphomas by array-CGH, we carefully analyzed more recent data from sequencing studies, assessing relevant 19p13 deletions and *LKB1* mutations in order to independently corroborate the potential role of *LKB1* as a driver event in B cell lymphoma (23–27). Analysis of 152 Diffuse Large B cell Lymphoma (DLBCL) by array comparative genomic hybridization (aCGH) shows evidence

of a homozygous deletion of the *LKB1*-containing region of 19p13 in one case, and single copy deletion in six other cases (Figure 1A, Supp. Table 1), all of which had a >50% decrease in expression of *LKB1* mRNA relative to the average expression level in DLBCL (27). Haploinsufficiency of *LKB1* has been shown to be oncogenic in murine models of melanoma and lung cancer (8, 28). aCGH analysis of DLBCL from another study has also identified GISTIC-defined 19p13.3 deletions in a significant number of lymphoma patients ($n = 15$, 8%) (22). Finally, analysis of published exome data revealed two missense mutations within the kinase domain of *LKB1* in DLBCL and B cell acute lymphoblastic leukemia (B-ALL) (Figure 1B) (12, 23). These results suggest that even though loss of *LKB1* may not be a common driver event of lymphoma in humans, it does occur at low frequency (0.1 to 5%).

Although *Lkb1* loss results in a dramatic increase in apoptosis and loss of HSC functions, the presence of *LKB1* mutations and deletions in patients with DLBCL and the well-established role of *LKB1* as a tumor suppressor in solid malignancies, suggest that *LKB1* may exhibit tumor suppressive properties in differentiated lymphocytes. In order to test this hypothesis, we crossed a murine *Lkb1*^{LoxP/LoxP} (*Lkb1*^{L/L}) conditional allele to either *CD19*^{Cre/+} (B cell) or Lck-Cre (T cell) respectively. *Lkb1* deletion in T cells resulted in a severe, lifelong decrease of peripheral blood T cells (Figure 2A and B) due to increased levels of apoptosis (Figure 2C), consistent with previously published data (16). Deletion of *Lkb1* in B cells had a similar effect, resulting in a reduction of B cells in all hematopoietic tissues, with 6-month old mice having almost no B cells in their peripheral blood (Figure 2D, Table 1). The lower number of B cells occurred in the setting of a markedly increased rate of apoptosis (Figure 2F) as in the *Lkb1*-deficient T-cell compartment, suggesting that *Lkb1* is necessary for survival of most lymphocytes. In the bone marrow, we observed changes in the distribution of B cell progenitors in young *CD19*^{Cre/+} *Lkb1*^{L/L} vs *CD19*^{Cre/+} *Lkb1*^{+/+} mice of about 8-weeks old. Specifically, *CD19*^{Cre/+} *Lkb1*^{L/L} exhibited increased numbers of pro and pre-B cells and decreased numbers of immature and mature re-circulating B cells (Figure 2G and H). This phenotype is consistent with a previous report (29), however, unlike that report, we did not observe significant changes in B cell sub-populations in the spleens of these mice at this young age (data not shown).

Despite the increased rate of apoptosis and resulting B cell deficiency, *CD19*^{Cre/+} *Lkb1*^{L/L} mice developed excess cancer starting at approximately one year of age (Figure 3A). Since many of the genetic events in humans that involve *LKB1* deletions are heterozygous, and that *Lkb1* haploinsufficiency has been shown to be oncogenic in other animal models, we also monitored a number of *CD19*^{Cre/+} *Lkb1*^{L/+} mice over time for signs of disease. Even though several cases of early lymphoma were observed, with the same immunophenotype as *CD19*^{Cre/+} *Lkb1*^{L/L} mice, the survival rate was not significantly different than control *CD19*^{Cre/+} mice (Figure 3A). Tumor-bearing *CD19*^{Cre/+} *Lkb1*^{L/L} mice exhibited splenomegaly, lymphadenopathy and thymic involvement with a monomorphic population of malignant B lymphocytes. These lymphomas consistently metastasized to the liver and lungs (Figure 3B, C, Supp. Figure 1A). Immunophenotypic analysis of the hematopoietic organs of tumor-bearing mice by flow cytometry revealed the presence of an expanded population of B220⁺, CD19⁺, IgM^{+/−}, Igλ[−], Igκ[−], CD43^{+/−}, Mac1^{low}, CD3[−] cells (Figure

3D and Supp. Figure 1B). Recombination of the *Lkb1* floxed allele was verified in all tumors and B cell populations as demonstrated in the spleen and lymph node in Figure 3E.

Lymphoma cells from CD45.2 *CD19^{Cre/+}Lkb1^{L/L}* mice were isolated and transplanted into sub-lethally irradiated CD45.1 recipient mice (Figure 3F). The presence of donor CD45.2 lymphoma cells in CD45.1 WT mice was assayed by flow cytometry, indicating that CD45.2 cells were readily transplantable in WT CD45.1 mice (Figure 3G–I), however, with an incomplete penetrance of around 50%. Clonality of the *Lkb1* null B cell lymphomas was assayed by PCR amplification of IgVH regions from B220+ splenocytes isolated from lymphoma-bearing animals. All lymphomas tested exhibited strong dominant bands in a background of a few weaker bands, suggesting that these lymphomas are predominantly monoclonal (Figure 3J).

Lkb1 regulates cell growth and energy metabolism partially via inactivation of the mTOR-mediated protein synthesis machinery. In order to test whether mTOR inhibition would therefore rescue the apoptotic phenotype in B cells, we treated *CD19^{Cre/+}Lkb1^{L/L}* mice with rapamycin, an mTOR inhibitor. Rapamycin treatment did not change the apoptotic rate of B cells in *CD19^{Cre/+}Lkb1^{L/L}* mice nor did it delay the onset of lymphoma (data not shown), suggesting protumorigenic activities of *Lkb1* loss in B cells does not depend on activation of the mTOR-pathway.

In contrast to deletion of *Lkb1* in B cells, deletion of *Lkb1* in T cells did not affect lifespan or increase the incidence of lymphoid malignancy (Figure 4A). These results show that *Lkb1* loss facilitates tumorigenesis of a subset of B but not T cells. In solid tumors, there is strong oncogenic cooperation between *Lkb1* loss and *Kras* activation (8, 30, 31). Since *Kras* activation functions as a strong survival signal, we hypothesized that expression of activated *Kras^{G12D}* might rescue lymphocyte apoptosis in the absence of *Lkb1* in lymphocytes, and thereby accelerate transformation. We therefore crossed the conditional *Lkb1^{L/L}* allele to the conditional LSL-*Kras^{G12D}* strain and monitored lymphocyte development (LSL=Lox Stop Lox). The LSL-*Kras^{G12D}* strain used has the *Kras^{G12D}* allele knocked into the endogenous *Kras* locus, downstream of a floxed stop cassette, allowing for conditional expression of the oncogenic allele (32). *Kras* activation in B cells did not rescue B cell apoptosis as a result of *Lkb1* loss (Figure 4B and C). *CD19^{Cre/+}Lkb1^{L/L}Kras^{G12D}* mice exhibited increased rates of apoptosis in B cells and also developed lymphoma with similar kinetics as mice with *Lkb1* loss alone (data not shown). *Kras* activation in T cells, however, produced a different effect. In young mice, the presence of activated *Kras^{G12D}* largely rescued T cell apoptosis and depletion in *Lck-Cre Lkb1^{L/L}* mice (Figure 4D and E). Long-term expression of *Kras^{G12D}* in the absence of *Lkb1* in T cells resulted in the development of T cell acute lymphoblastic leukemia (T-ALL), with an immunophenotype identical to mice with *Kras^{G12D}* activation alone. Mice exhibited elevated WBC counts, enlarged spleens, thymus and lymph nodes (Figure 4F) and were completely taken over by CD4⁺CD8⁺ double-positive cells in all hematopoietic organs (Figure 4G–I). Median cancer free survival of *Lck-Cre Lkb1^{L/L}Kras^{G12D}* mice was 137 days (Figure 4A), lower than that of *Lck-Cre Lkb1^{L/+}Kras^{G12D}* (33), but not significantly different. Despite its pro-apoptotic effect, *Lkb1* loss in T cells was therefore not sufficient to prevent the onset of *Kras*-mediated T-ALL. Moreover, these

results did not provide evidence for cooperation between RAS activation and LKB1 inactivation in lymphomagenesis.

DISCUSSION

The role of *Lkb1* as a tumor suppressor and its function during metastasis has been extensively studied in a variety of solid tumors, however, the role of *Lkb1* in the development of hematopoietic malignancies is much less clear. Several studies have demonstrated that *Lkb1* is necessary for HSC survival, a phenotype that is not consistent with a tumor suppressive function. On the other hand, array-CGH and other sequencing studies have identified *Lkb1* deletions and mutations in B cell lymphomas, suggesting a potential tumor suppressive role for *Lkb1* in lymphoma. Deletions of the 19p13.3 region, which includes *LKB1*, in B cell lymphomas have been identified as far back as 1994, however, due to the size of these deletions, it has been a challenge to identify which gene or genes may be responsible for promoting malignant transformation. Given the role of *Lkb1* as a tumor suppressor in solid tumors and the multiple studies that implicated 19p13 deletions in lymphoma, we wanted to clarify whether *Lkb1* would function as a tumor-suppressor in lymphocytes. Overall, our results show that deletion of *Lkb1* does promote the development of B cell lymphoma in mice, suggesting that loss of LKB1 in humans with 19p13 deletions could be one of the driving oncogenic events.

Given the size of these deletions in chromosome 19p13, it is likely that deletion of *LKB1* is not the only driver event, as other genes within this region have also been implicated in carcinogenesis. We were not able, however, to identify any other common genetic events in the seven cases of LKB1 deletion in DLBCL. Furthermore, the small number of cases with point mutation and the fact that there is insufficient genomic information for these few cases, it is hard to determine whether these events cooperate with other oncogenic events to drive lymphomagenesis. Given the size of some of these deletion, perhaps in future studies it would be of interest to investigate whether combinatorial inactivation of multiple genes within the 19p13.3 region would cooperate in accelerating the onset of lymphoma.

Previous studies have also investigated the effects of tissue-specific *Lkb1* deletion during B and T lymphocyte development (16, 29). Specifically, Walsh et al (29) investigated how B cells regulate T cell-dependent antigenic stimulation and differentiation into plasma and memory B cells. They suggested that *Lkb1* expression is required to maintain B cell quiescence and prevent the premature formation of germinal centers (GCs), however, they did not report any increased incidence of lymphoma after deletion of *Lkb1* (29). Our results are consistent with most of the observations in this study, with no dramatic phenotype in B cell development other than decreased numbers of immature and recirculating B cells in the bone marrow (Figure 2G–H). Differences in the studies could be attributed to variations in experimentation and timing of experiments. While the previous report only studied animals up to 12 weeks old, they did observe an expansion of GCs upon deletion of *Lkb1*. This could perhaps be an early sign of early neoplasia and had the mice been monitored longer, they would have developed lymphoma. In our studies, after monitoring a cohort of mice for almost two years, we observed a massive induction of apoptosis within the B cell lineage in all hematopoietic tissues upon *Lkb1* deletion (Figure 2A) and mice developed lymphoma

with a median survival of about one year (Figure 3A). In T lymphocytes, Maciver et al reported defects in proliferation and viability after deletion of *Lkb1* using a T cell-specific Cre driver (16). Our results are consistent with this report as we observe a massive increase in T cell apoptosis upon deletion of *Lkb1* (Figure 1A). Interestingly, even though *Lkb1* deletion in B cells resulted in the development of lymphoma, *Lkb1* deletion in T cells, as a single event, was not sufficient to drive cancer formation. Even though *Lkb1* deletion potentially cooperates with Kras activation to drive cancer formation in solid tumors, Kras activation in B cells did not rescue B cell apoptosis nor accelerate the onset of lymphoma. In contrast, Kras activation in T cells did rescue *Lkb1*-deficient cells from apoptosis, however, it did not accelerate the onset of T-ALL. The differential effect of Kras activation on B vs T cells is intriguing and highlights the different regulatory networks for essential biological functions, such as survival, even in such similar cell types like lymphocytes. It also highlights the fact that cancer initiation mechanisms vary extensively even in related cell types within the hematopoietic system.

Our results also highlight another potential paradox, on how excessive apoptosis could promote tumorigenesis. Increased proliferation coupled to decreased cell death is one of the hallmarks of cancer, where cancer cells circumvent apoptotic response pathways, preventing their elimination. Apoptosis resistance by itself is often not sufficient to promote cancer development and is often coupled with other genetic or epigenetic events to promote transformation. However, continuous apoptosis, as in the case of *Lkb1* deletion in B cells, could become a driver event, promoting constant regeneration of a niche, increasing the risk for clonal selection of transformed B cell clones with a growth competitive advantage.

In summary, using murine genetic studies, we have identified *Lkb1* as a tumor suppressor in B cell lymphoma, consistent with the presence of 19p13 deletions and low frequency *LKB1* mutations in human patients with DLBCL. In accord with this finding, our murine studies suggest that *Lkb1* potentially represses B cell transformation, despite also playing a strong pro-survival role in most lymphocytes. Neither the survival nor transformability of *Lkb1*-deficient B cells is enhanced by excess RAS activation. In contrast, T cells are not susceptible to transformation by *Lkb1* deletion. This report demonstrates a paradoxical role for *LKB1* in human lymphocytes, promoting the survival of B and T cells, while also preventing the transformation of B cells. In aggregate, these results establish that complete *LKB1* inactivation in B cell lymphoma could be the driver oncogenic event in 19p13 deletions that are observed in B cell lymphomas. Given the disparate functions of *LKB1* in metabolism, metastatic spread, polarity and cell survival (13, 15, 28, 34), it seems like that lymphoma driven by *LKB1* inactivation may also differ significantly from other types of B cell lymphoma in terms of therapeutic response, clinical course and prognosis.

METHODS

Animals

Animals were housed in an AAALAC-accredited facility and treated in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) for animal research of the University of North Carolina at Chapel Hill. The conditional *Lkb1*

floxed allele is described here (35) and generation of the LSL-Kras^{G12D} allele is described here (32).

Blood counts and Flow cytometry analysis

Blood was collected using heparinized capillary tubes and analyzed on a Hemavet Analyzer (Drew Scientific). For Flow cytometry analysis, spleen cells or peripheral blood were made into a single cell suspension, washed with HBSS+2%FBS and stained with the appropriate antibodies. Cells were analyzed in HBSS+2% FBS and 0.4ug/ml propidium iodide. Flow cytometry analysis was performed on Cyan (Beckman Coulter) and LSRII (BD) flow cytometers. Flow cytometry data were analyzed using FlowJo software (TreeStar Inc.). For apoptosis assays, cells were prepared as described in the Annexin-V kit (Life Technologies).

Lymphoma transplants

CD19⁺ lymphoma cells were sorted from lymphoma-bearing CD45.2 *CD19^{Cre/+}Lkb1^{L/L}* mice by magnetic bead sorting using the AutoMACS (Miltenyi Biotech). Cells were washed and resuspended in HBSS. 500,000 cells were transplanted into sub-lethally irradiated recipient CD45.1 mice. Recipient mice were analyzed for the presence of lymphoma cells in the peripheral blood at 4 weeks post-transplant and at 4 week intervals after that.

Histology

Spleens and livers from tumor-bearing animals was harvested, placed in 10% formalin overnight and transferred to 70% ethanol until sectioning. The tissue was embedded in paraffin, sectioned at 5 m and stained with hematoxylin and eosin (H&E) for histopathologic assessment. Histopathology slides were reviewed by a board-certified hematopathologist (Y.F.).

Antibodies used for Flow cytometry

CD45R/B220 (clone RA3-6B2, 1:100, eBioscience), CD3 (145-2c11, 1:100, eBioscience), CD45.2 (clone 104, 1:100, eBioscience), CD4 (clone GK1.5, 1:100, eBioscience), CD8 (clone 53-6.7, 1:100, eBioscience), CD19 (clone eBio1D3, 1:100, eBioscience), CD5 (clone 53-7.3, 1:100, eBioscience), CD21 (clone eBio4E3, 1:100, eBioscience), CD43 (clone eBioR2/60, 1:100, eBioscience), IgD (clone 11-26, 1:100, eBioscience), Gr1 (clone RB6-8C5, 1:100, eBioscience), IgM (clone 11f41, 1:100, eBioscience), GL-7 (clone GL-7, 1:100, eBioscience), Mac1 (clone M1/70, 1:100, eBioscience).

Deletion and mutation analysis

Lymphoma sequencing studies from Lenz et. al. (27) were reanalyzed to identify deletions that included the LKB1 locus. Identification of somatic LKB1 mutations was carried out by analyzing tumor sequencing data from cBioportal (23, 25).

Clonality and IgVH Rearrangement Analysis

Clonality of lymphomas was determined by assessing IgVH rearrangements on cDNA isolated from CD19⁺ lymphoma cells from the spleen or lymph node of *CD19^{Cre}Lkb1^{L/L}* mice. PCR primers identify the framework region of the most abundant IgVH gene family

and JH1-4 segments (36). Sense primers, VHJ558: 5'-CGAGCTCTCCARCACAGCCTWCATGCARCTCARC-3' VH7183: 5'-CGGTACCAAGAASAMCCTGTWCCTGCAAATGASC-3' VHQ52: 5'-CGGTACCAGACTGARCATCASCAAGGACAAYTCC-3' VHGam3.8: 5'-CAAGGGACGGTTTGCCTTCTCTTTGGAA-3'. Anti-sense primer, JH4: 5'-TCTCAGCCGGCTCCCTCAGGG-3'. PCR was performed using the standard protocol for Phusion polymerase (New England Biolabs) with 50ng cDNA template and samples were run on a 1.5% agarose gel.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Hemminki A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*. 1998; 391(6663):184–187. [PubMed: 9428765]
2. Alessi DR, Sakamoto K, Bayascas JR. LKB1-dependent signaling pathways. *Annu Rev Biochem*. 2006; 75(1):137–163. [PubMed: 16756488]
3. Shah U, Sharpless NE, Hayes DN. LKB1 and Lung Cancer: More Than the Usual Suspects. *Cancer Res*. 2008; 68(10):3562–3565. [PubMed: 18483235]
4. Hearle N. Frequency and Spectrum of Cancers in the Peutz-Jeghers Syndrome. *Clinical Cancer Research*. 2006; 12(10):3209–3215. [PubMed: 16707622]
5. van Lier MGF, et al. High Cancer Risk in Peutz-Jeghers Syndrome: A Systematic Review and Surveillance Recommendations. *The American Journal of Gastroenterology*. 2010; 105(6):1258–1264. [PubMed: 20051941]
6. Sanchez-Cespedes M, et al. Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. *Cancer Res*. 2002; 62(13):3659–3662. [PubMed: 12097271]
7. Avizienyte E, et al. LKB1 somatic mutations in sporadic tumors. *AJPA*. 1999; 154(3):677–681.
8. Ji H, et al. LKB1 modulates lung cancer differentiation and metastasis. *Nature*. 2007; 448(7155):807–810. [PubMed: 17676035]
9. Wingo SN, et al. Somatic LKB1 Mutations Promote Cervical Cancer Progression. *PLoS ONE*. 2009; 4(4):e5137–8. [PubMed: 19340305]
10. Rowan A, et al. Somatic Mutations in the Peutz-Jeghers (LKB1/STKII) Gene in Sporadic Malignant Melanomas. *Journal of Investigative Dermatology*. 1999; 112(4):509–511. [PubMed: 10201537]
11. Guldberg P, et al. Somatic mutation of the Peutz-Jeghers syndrome gene, LKB1/STK11, in malignant melanoma. *Oncogene*. 1999; 18(9):1777–1780. [PubMed: 10208439]
12. Forbes SA, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res*. 2015; 43(D1):D805–D811. [PubMed: 25355519]
13. Nakada D, Saunders TL, Morrison SJ. Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells. 2010; 468(7324):653–658.
14. Gurumurthy S, et al. The Lkb1 metabolic sensor maintains haematopoietic stem cell survival. *Nature*. 2010; 468(7324):659–663. [PubMed: 21124451]
15. Gan B, et al. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. *Nature*. 2010; 468(7324):701–704. [PubMed: 21124456]

16. Maciver NJ, et al. The Liver Kinase B1 Is a Central Regulator of T Cell Development, Activation, and Metabolism. *The Journal of Immunology*. 2011;1–13.
17. Yoshioka T, et al. Cytogenetic features of de novo CD5-positive diffuse large B-cell lymphoma: Chromosome aberrations affecting 8p21 and 11q13 constitute major subgroups with different overall survival. *Genes Chromosom Cancer*. 2005; 42(2):149–157. [PubMed: 15543600]
18. Karnan S, et al. Analysis of chromosomal imbalances in de novo CD5-positive diffuse large-B-cell lymphoma detected by comparative genomic hybridization. *Genes Chromosom Cancer*. 2003; 39(1):77–81.
19. Halldórsdóttir AM, et al. High-resolution genomic screening in mantle cell lymphoma-specific changes correlate with genomic complexity, the proliferation signature and survival. *Genes Chromosom Cancer*. 2010; 50(2):113–121.
20. Mao X, et al. Comparative genomic hybridization analysis of primary cutaneous B-cell lymphomas: Identification of common genomic alterations in disease pathogenesis. *Genes Chromosom Cancer*. 2002; 35(2):144–155. [PubMed: 12203778]
21. Scholtysik R, et al. Recurrent deletions of the TNFSF7 and TNFSF9 genes in 19p13.3 in diffuse large B-cell and Burkitt lymphomas. *Int J Cancer*. 2012; 131(5):E830–E835. [PubMed: 22213068]
22. Monti S, et al. Integrative Analysis Reveals an Outcome-Associated and Targetable Pattern of p53 and Cell Cycle Deregulation in Diffuse Large B Cell Lymphoma. *Cancer Cell*. 2012; 22(3):359–372. [PubMed: 22975378]
23. Cerami E, et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. *Cancer Discovery*. 2012; 2(5):401–404. [PubMed: 22588877]
24. Forbes S, et al. COSMIC 2005. *Br J Cancer*. 2006; 94(2):318–322. [PubMed: 16421597]
25. Gao J, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling*. 2013; 6(269):p11–p11. [PubMed: 23550210]
26. Roberts KG, et al. Targetable Kinase-Activating Lesions in Ph-like Acute Lymphoblastic Leukemia. *N Engl J Med*. 2014; 371(11):1005–1015. [PubMed: 25207766]
27. Lenz GG, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci USA*. 2008; 105(36):13520–13525. [PubMed: 18765795]
28. Liu W, et al. LKB1/STK11 Inactivation Leads to Expansion of a Prometastatic Tumor Subpopulation in Melanoma [Internet]. *Cancer Cell*. 2012; 21(6):751–764. [PubMed: 22698401]
29. Walsh NC, et al. LKB1 inhibition of NF- κ B in B cells prevents T follicular helper cell differentiation and germinal center formation. *EMBO Rep*. 2015; 16(6):753–768. [PubMed: 25916856]
30. Matsumoto S, et al. Prevalence and specificity of LKB1 genetic alterations in lung cancers. *Oncogene*. 2007; 26(40):5911–5918. [PubMed: 17384680]
31. Morton JP, et al. LKB1 Haploinsufficiency Cooperates With Kras to Promote Pancreatic Cancer Through Suppression of p21-Dependent Growth Arrest. *Gastroenterology*. 2010; 139(2):586–597.e6. [PubMed: 20452353]
32. Tuveson DA, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell*. 2004; 5(4):375–387. [PubMed: 15093544]
33. Chiang MY, Xu L, Shestova O, Histen G. Leukemia-associated NOTCH1 alleles are weak tumor initiators but accelerate K-ras-initiated leukemia. *The Journal of ....* [published online ahead of print: 2008];
34. Gurumurthy S, Hezel AF, Berger JH, Bosenberg MW, Bardeesy N. LKB1 Deficiency Sensitizes Mice to Carcinogen-Induced Tumorigenesis. *Cancer Res*. 2008; 68(1):55–63. [PubMed: 18172296]
35. Bardeesy N, et al. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature*. 2002; 419(6903):162–167. [PubMed: 12226664]
36. Hanna J, et al. Direct Reprogramming of Terminally Differentiated Mature B Lymphocytes to Pluripotency. *Cell*. 2008; 133(2):250–264. [PubMed: 18423197]

Figure 1. *LKB1* deletions and missense mutations in human B cell lymphomas

(A) Seven cases of patients with B cell lymphoma from aCGH study with homozygous or heterozygous *LKB1* deletion and with at least 50% reduction in expression (from Lenz et. al) (27). ABC = Activated B cell lymphoma, GCB = Germinal Center B cell lymphoma, PMBL = Primary Medistial B cell lymphoma

(B) Missense mutations in the kinase domain of *LKB1* in two patients, one with DLBCL and one with BALL. Data analysis through COSMIC (Forbes et. al) (12).

Figure 2. *Lkb1* deletion in lymphocytes induces apoptosis and perturbs B cell development

(A) Flow cytometry analysis of hematopoietic lineage distribution from the peripheral blood of *Lck-Cre* and *Lck-Cre Lkb1^{L/L}* mice. Black squares indicate CD4/8+ T cells.

(B) Absolute cell numbers of lymphocytes in the peripheral blood of *Lck-Cre Lkb1^{+/+}* and *Lck-Cre Lkb1^{L/L}* mice calculated using complete blood counts and flow cytometry analysis. (n = 5)

(C) Steady state apoptosis levels of lymphocytes using Annexin-V in *Lck-Cre Lkb1^{+/+}* and *Lck-Cre Lkb1^{L/L}* mice. (n = 5)

(D) Flow cytometry analysis of hematopoietic lineage distribution from the peripheral blood of young 6–8-week old *CD19^{Cre/+} Lkb1^{+/+}* and *CD19^{Cre/+} Lkb1^{L/L}* mice. Black ovals indicate B220+ B cells. 6-mo = six-month old mice.

(E) Absolute cell numbers of lymphocytes in the peripheral blood of *CD19^{Cre/+} Lkb1^{+/+}* and *CD19^{Cre/+} Lkb1^{L/L}* mice calculated using complete blood counts and flow cytometry analysis. (n = 5)

(F) Steady state apoptosis levels of lymphocytes using Annexin-V in *CD19^{Cre/+} Lkb1^{+/+}* and *CD19^{Cre/+} Lkb1^{L/L}* mice. (n = 5)

(G) Flow cytometry analysis of B cell progenitors in the bone marrow of *CD19^{Cre/+} Lkb1^{L/L}* and *CD19^{Cre/+} Lkb1^{+/+}* control animals. Immature B cells = B220+, IgM-. Mature

circulating B cells = B220^{high}, IgM+. Pre-B = B220+, IgM-, CD19+, CD43-. Pro-B = B220+, IgM-, CD19+, CD43+. Pre-proB = B220+, IgM-, CD19-, CD43-. Frequencies of the pre-, pro- and pre-pro B cell populations are for the parent gate of B cell progenitors (B220+, IgM-).

(H) Quantitative analysis of the data in **G** (n = 5).

Figure 3. *Lkb1* deletion in lymphocytes promotes formation of B cell lymphoma

(A) Kaplan-Meier cancer free survival curve of $CD19^{Cre/+} Lkb1^{L/L}$ ($n = 18$). Median survival is 400 days ($P < 0.001$).

(B) Enlarged spleens from $CD19^{Cre/+} Lkb1^{L/L}$ mice with lymphoma. Distinct tumors are also present in the spleen, liver and lung.

(C) H&E staining of organs from $CD19^{Cre/+} Lkb1^{L/L}$ mice with lymphoma showing disrupted architecture of the spleen (left) and lymphocyte infiltration in the liver (right). (D) Flow cytometry analysis of cells isolated from the spleen of a representative tumor-bearing $CD19^{Cre/+} Lkb1^{L/L}$ mouse. Lymphoma cells are indicated in the black oval and are B220 positive.

(E) PCR screening for deletion of *Lkb1* floxed locus using DNA from malignant B220+ B cells. LN = Lymph node, S = Spleen, Ctrl = negative control, A and B = two different cases of lymphoma, 1:10 indicates DNA dilution.

(F) Schematic of adoptive transfer of donor $CD45.2$ lymphoma cells to $CD45.1$ secondary WT recipient mice ($n = 7$).

(G-I) Flow cytometry analysis of the peripheral blood (G), bone marrow (H) spleen of non-transplanted mice vs mice transplanted with B220+ lymphoma cells from F.

(J) Clonality analysis for IgVH rearrangements, performed by PCR on B220+ splenocytes. Lanes 1–8 indicated 8 different lymphomas. Lane 9 indicates non-malignant B cells from the spleen of a wild type mouse. Arrows on lane 9 indicate the presence of multiple clones in wild type B cells. Stars (*) indicate the predominant clones in each lymphoma. Throughout, error bars indicate s.e.m. ** $P < 0.01$

Figure 4. Activated *Kras^{G12D}* rescues apoptotic T cells but not B cells after *Lkb1* deletion

(A) Kaplan-Meier analysis of cancer free survival. Median survival of *Lck-Cre Lkb1^{L/L} Kras^{G12D}* mice was 133 days ($P < 0.001$, compared to *Lck-Cre Lkb1^{+/+}*) and for *Lck-Cre Lkb1^{L/+} Kras^{G12D}* mice 169 days.

(B) Flow cytometry analysis of hematopoietic lineage distribution from the peripheral blood of mice with the indicated genotypes. Black ovals indicate B220+ B cells.

(C) Quantitative analysis and calculation of absolute numbers of lymphoid and myeloid lineages in the peripheral blood from the indicated genotypes.

(D) Flow cytometry analysis of hematopoietic lineage distribution from the peripheral blood of mice with the indicated genotypes. Black squares highlight the T cell populations, demonstrating recovery of the T cell compartment in mice lacking *Lkb1* in the presence of activated *Kras^{G12D}*.

(E) Quantitative analysis of the T cell population in the peripheral blood from panel D. (PB = peripheral blood).

(F) Enlarged spleen and lymph nodes from disease-bearing *Lck-Cre Lkb1^{L/L} Kras^{G12D}* mice vs *Lck-Cre* control mice.

(G) Flow cytometry analysis of thymocytes for T cell populations from *Lck-Cre Lkb1^{+/+}* and *Lck-Cre Lkb1^{L/L}* mice. Leukemic T cells are CD4+, CD8+, CD25+ and CD44-.

(H) Flow cytometry analysis of hematopoietic lineages from the bone marrow of *Lck*-Cre *Lkb1*^{+/+} and the disease-bearing *Lck*-Cre *Kras*^{G12D} and *Lck*-Cre *Lkb1*^{L/L} *Kras*^{G12D} mice. Black squares indicate the expansion of T cells at the expense of other lineages.

(I) Flow cytometry analysis of spleen cells isolated from *Lck*-Cre *Lkb1*^{+/+} and the disease-bearing *Lck*-Cre *Kras*^{G12D} and *Lck*-Cre *Lkb1*^{L/L} *Kras*^{G12D} mice. Black squares indicate the leukemic CD4⁺ CD8⁺ T cells.

Error bars indicate s.e.m throughout.

Table 1 Complete blood count comparison between *CD19^{Cre}* control and *CD19^{Cre}Lkb1^{L/L}*

	<i>CD19^{Cre}</i>	<i>CD19^{Cre}Lkb1^{L/L}</i>
White Blood Cells, K/ μ l	9.2 $\pm$ 1.71	1.96 $\pm$ 1.13
Red Blood Cells, M/ μ l	8.92 $\pm$ 0.49	10.65 $\pm$ 0.60
Hemoglobin, g/dL	12.12 $\pm$ 0.63	13.80 $\pm$ 0.7
Hematocrit, %	41.23 $\pm$ 0.41	46.40 $\pm$ 2.19
MCV, fL	50.26 $\pm$ 0.63	43.60 $\pm$ 0.73
MCH, Pg	14.74 $\pm$ 0.24	12.96 $\pm$ 0.27
MCHC, g/dL	29.85 $\pm$ 0.56	29.74 $\pm$ 0.62
Platelets, K/ μ L	958 $\pm$ 42	615 $\pm$ 83

Table 2

Deletion events that include STK11

Sample name3	Lymphoma subgroup	Segment number	chromosome	hg17 loc.start	hg17 loc.end	#probes	Raw copy number	Copy Change	hg17 genomic coordinates	hg19 genomic coordinates
2001	ABC	6352	19	50250	19639551	2579	0.400871292	Homozygous deletion	chr19:50250-19639551	chr19:99250-19778551
704	ABC	44538	19	50250	2475061	256	0.674378034	Heterozygous deletion	chr19:50250-2475061	chr19:99250-2524061
726	ABC	22326	19	50250	19649331	2580	1.396786488	Heterozygous deletion	chr19:50250-19649331	chr19:99250-19788331
1026	GCB	42369	19	50250	63789421	7020	1.346705017	Heterozygous deletion	chr19:50250-63789421	Unable to convert
1991	GCB	5650	19	50250	12171874	1558	0.936115927	Heterozygous deletion	chr19:50250-12171874	chr19:99250-12310874
618	GCB	44064	19	50250	11956583	1528	1.147088266	Heterozygous deletion	chr19:50250-11956583	chr19:99250-12095583
701	GCB	20538	19	321978	4146370	471	0.910467154	Heterozygous deletion	chr19:321978-4146370	chr19:370978-4195370
1992	PMBL	5958	19	50250	20533019	2636	1.221315666	Heterozygous deletion	chr19:50250-20533019	chr19:99250-20741179

Sample id	Lymphoma type	AA mutation	CDS mutation	Type	Reference
COSS2479247	DLBCL	p.L184F	c.550C>T	Missense	PMID: 24531327
COSS2364168	ALL	p.P280S	c.838C>T	Missense	PMID: 25207766
2426603	Plasma Cell Myeloma	p.P281fs*6		frame shift	PMID: 24335104