RESEARCH ARTICLE

# The Prognostic Value of Decreased LKB1 in Solid Tumors: A Meta-Analysis

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## Abstract

### Background

Liver kinase B1 (LKB1) is a protein kinase that regulates the growth, integrity and polarity of mammalian cells. Recent studies have reported the prognostic value of decreased LKB1 expression in different tumors. However, the results of these studies remain controversial. Therefore, this meta-analysis was performed to more accurately estimate the role of decreased LKB1 in the prognostication of human solid tumors.

### Methods

A systematic literature search in the electronic databases PubMed, Embase, Web of Science and CNKI (updated to October 15, 2015) was performed to identify eligible studies. The overall survival (OS), relapse-free survival (RFS), disease-free survival (DFS) and clinicopathological features data were collected from these studies. The hazard ratios (HRs), odds ratios (ORs) and 95% confidence intervals (CIs) were calculated and pooled with a random-effects models using Stata12.0 software.

### Results

A total of 14 studies covering 1915 patients with solid tumors were included in this meta-analysis. Decreased LKB1 was associated with poorer OS in both the univariate (HR: 1.86, 95%CI: 1.42–2.42, P<0.001) and multivariate (HR: 1.55, 95%CI: 1.09–2.21, P = 0.015) analyses. A subgroup analysis revealed that the associations between decreased LKB1 and poor OS were significant within the Asian region (HR 2.18, 95%CI: 1.66–2.86, P<0.001) and obvious for lung cancer (HR: 2.16, 95%CI: 1.47–3.18, P<0.001). However, the articles that involved analyses of both RFS and DFS numbered only 3, and no statistically significant correlations of decreased LKB1 with RFS or DFS were observed in this study. Additionally, the pooled odds ratios (ORs) indicated that decreased LKB1 was associated with larger tumor size (OR: 1.60, 95%CI: 1.09–2.36, P = 0.017), lymph node

metastasis (OR: 2.41, 95%CI: 1.53–3.78, P<0.001) and a higher TNM stage (OR: 3.35, 95%CI: 2.20–5.09, P<0.001).

## Conclusion

These results suggest that decreased LKB1 expression in patients with solid tumors might be related to poor prognosis and serve as a potential predictive marker of poor clinicopathological prognostic factors. Additional studies are required to verify the clinical utility of decreased LKB1 in solid tumors.

## Introduction

Liver kinase B1 (LKB1) is a protein kinase also known as serine/threonine kinase 11 that is encoded by the STK11 gene in humans [1]. LKB1 is the homologue of par-4 in non-mammalian species [2] and can regulate early embryonic development in both mammals and non-mammals [2–4]. LKB1 has been linked to the regulation of epithelial integrity and polarity [5,6]. The loss of LKB1 disrupts epithelial cell polarity and promotes cancer progression, invasion and metastasis [7,8]. Experimental evidence also indicates that LKB1 deficiency can cause adenocarcinomas to transdifferentiate into squamous cell carcinomas [9]. Therefore, LKB1 is considered a tumor suppressor kinase [10].

Studies have demonstrated that low LKB1 protein expression is associated with worse overall survival (OS) in human breast cancer [11]. Additionally, low LKB1 expression levels in human pancreatic ductal adenocarcinomas and decreased expression of LKB1 in hepatocellular carcinoma patients are poor prognostic factors [12,13]. Reports continue to suggest that LKB1 loss at the protein level plays a role in the poor outcomes of patients with colorectal cancer and non-small cell lung cancer [14,15]. Moreover, studies have also indicated that low expression of LKB1 is associated with tumor clinicopathological features [15,16].

Although some evidence suggests that decreased LKB1 is an important factor that is implicated in poorer survival in solid tumor patients [11–16], some conflicting results have also been reported [17,18]. However, these results still seem to be controversial. Consequently, we initiated a meta-analysis to determine the significance of decreased LKB1 expression in the prediction of clinical outcomes and to examine the association between decreased LKB1 and the clinicopathological parameters of solid tumors.

## Materials and Methods

### Literature Search Strategy

The literature relevant to LKB1 expression and survival in solid tumors was searched in the PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI) databases through October 15, 2015. The search terms included the following key words in various combinations: LKB1, STK11, liver kinase b1, prognosis, prognostic, survival, and overall survival. The list of publications was limited to human studies and restricted to those published in Chinese or English. The references of the review articles and primary research were further searched to identify additional potentially relevant studies to avoid omission due to the electronic search approach.

## Study Inclusion and Exclusion Criteria

The studies that were included in this meta-analysis met the following criteria: (1) a pathological diagnosis of cancer was made;(2) original published studies with full text that measured LKB1 protein expression in patients with any type of tumor via immunohistochemistry or western blotting; (3) associations of LKB1 expression with overall survival (OS), relapse-free survival (RFS), disease-free survival (DFS), or clinicopathological features were described; (4) hazard ratios (HRs) and 95% confidence intervals (CIs) were reported or could be calculated based on the information in the paper; and (5) when the same author reported repeated results from the same population, the most complete report was included.

The exclusion criteria for this meta-analysis were as follows: (1) unpublished papers; (2) laboratory articles, review and letters; (3) articles with only animal experiments; and (4) studies without information about survival outcomes or survival curves and those in languages other than Chinese and English.

## Quality Assessment

Two independent reviewers (Xi Chen and Xiaoxiao Lu) scored the qualities of the selected papers using the Newcastle—Ottawa Quality Assessment Scale (NOS), which was referenced in a previously published paper [19] (S1 Table). Briefly, the score of each paper was decided based on selection, comparability and outcome according to the NOS. Each appraised study received a score between 0 and 9. NOS scores of 9–7, 6–4 and 3–1 were defined as high-, intermediate- and low-quality studies, respectively. Discrepancies were discussed until a consensus was reached regarding the final score for each paper.

## Data Extraction

For the eligible studies, two investigators (Ying Gao and Wei Li) independently extracted the following data: first author's name, publication year, region, type of cancer, number of patients, patients' sexes and ages, follow-up times, test methods, staining positions, cut-off values, survival data (including OS, RFS or DFS), analysis method, and clinicopathological parameters, such as tumor differentiation, tumor size/invasion depth, lymph node metastasis and TNM stage. For studies that presented only Kaplan-Meier curves, Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/, a free down-loaded software) was used to extract the survival data. The estimates of the HRs and 95% CIs were calculated by Tierney's method as previously described [20]. Subsequently, the raw data were entered into GraphPad Prism 6.0 (GraphPad Software, Inc.) to produce Kaplan-Meier curves for comparison with the published curves [21]. Any disagreements were adjudicated by discussion until a consensus was reached.

## Statistical Analysis

This meta-analysis was performed using Stata 12.0 (Stata Corporation, College Station, TX, USA) software. Generic inverse variance weighting was used to pool the HRs. When the result of a Q-test ($I^2 > 50\%$ or $P < 0.05$) indicated heterogeneity between the studies, the random-effects model was used for the meta-analysis. Otherwise, a fixed-effects model was used [22]. An HR greater than 1 indicated poor prognosis in patients with decreased LKB1. The chi-squared test (Cochrane's Q test) and I-squared statistical test were used to analyze the heterogeneity between studies. A sensitivity analysis was used to test the influences of individual studies on the pooled HR to evaluate the stability of the meta-analysis. Because unequal characteristics might have been included in the eligible studies, subgroup stratification analyses were performed according to the testing method, region, cancer type, staining position and analysis

method to identify the sources of heterogeneity. Funnel plots were used to graphically represent the publication bias. Begg's (rank correlation) and Egger's (regression asymmetry) tests were adopted to confirm the publication bias.

Pooled estimates of the odds ratios (OR) were calculated using the Mantel-Haenszel method to estimate the correlations of LKB1 expression with the clinicopathological parameters, which included tumor differentiation, tumor size, lymph node metastasis and TNM stage. ORs greater than 1 indicated that decreased LKB1 expression was likely related to poor differentiation, large tumor size (or deep invasion), lymph node metastasis and advanced TNM stage. P-values<0.05 were considered statistically significant.

## Results

### Study Search Information

The initial search identified one hundred and eleven potentially relevant titles. A further review of the screening results revealed that seventeen studies were of acceptable relevance for retrieval of the full text. However, two of these studies were excluded because the survival curves were based on LKB1 gene expression [23,24], and one additional study was ruled out because the specimens were metastatic tumors [25]. Ultimately, fourteen studies [11–18,26–31] met the eligibility criteria and were included in the current meta-analysis (Fig 1).
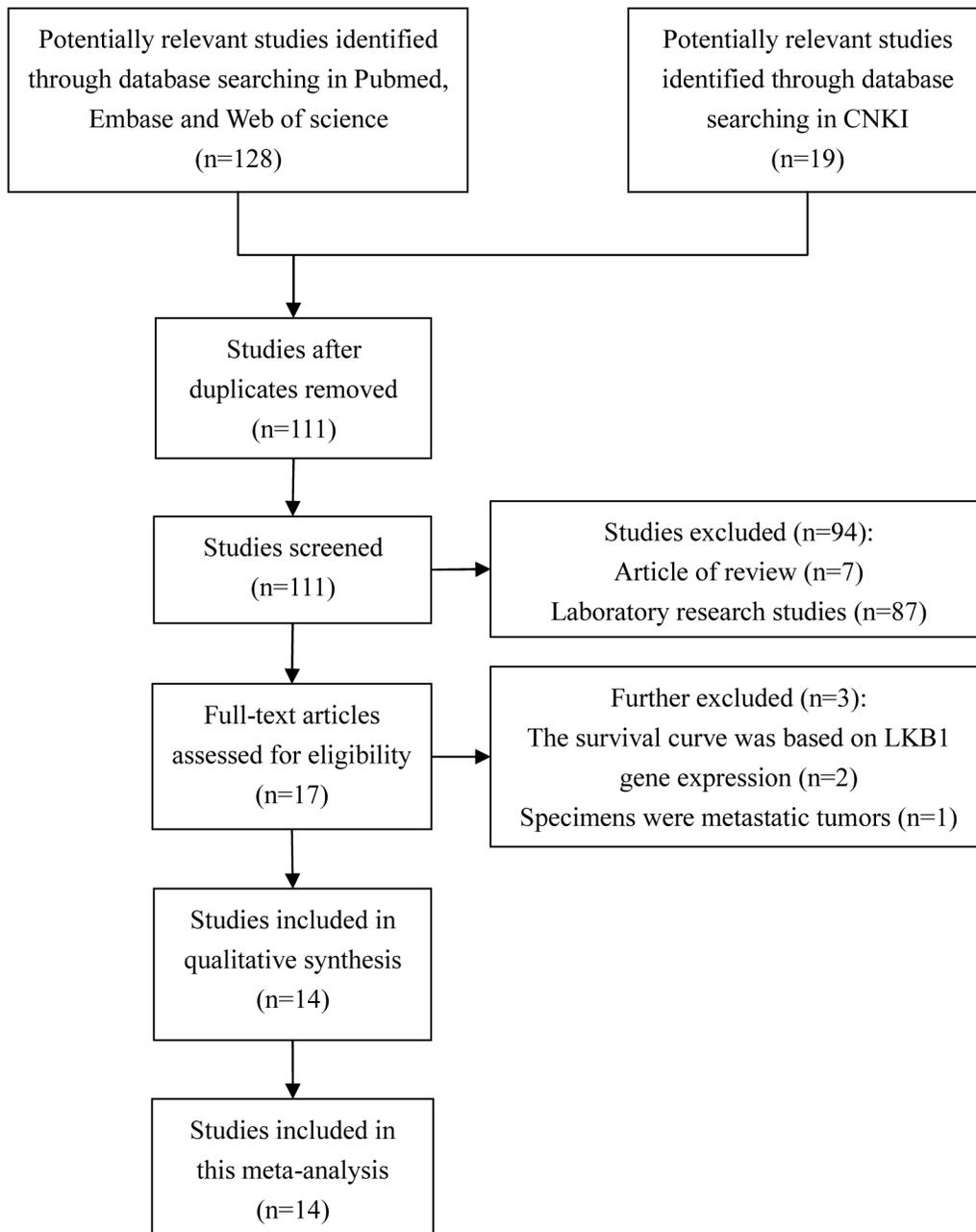
### Description of the Studies

The characteristics of the 14 identified studies are shown in Tables 1 and 2. In total, 1915 patients from five regions (China, Taiwan, the USA, France and the UK) were included in these studies. The solid tumors that were included in this meta-analysis were derived from the following seven cancer types: lung adenocarcinomas (or non-small cell lung cancers), breast carcinomas, gastric cancers, hepatocellular carcinomas, pancreatic cancers (or pancreatic ductal adenocarcinomas), colorectal cancers, and intrahepatic cholangiocarcinomas. The NOS scores of these studies ranged from 5 to 8 (mean: 6.33; S1 and S2 Tables), thus, the studies were of high quality.

### Decreased LKB1 Expression and OS

The pooled HR values revealed that decreased expression of LKB1 protein was significantly associated with OS in relation to solid tumors (HR: 1.86, 95%CI: 1.42–2.42, P<0.001; Fig 2). Additionally, significant heterogeneity ($I^2$ = 73.50%, P<0.001) was observed when using a random-effects model to analyze the pooled HR values of the OSs. By successively omitting each study from the aggregated survival meta-analyses, a sensitivity analysis was performed to evaluate the influence of each individual study on the pooled HR. The results revealed that the pooled estimates of the effect of decreased LKB1 expression on the OS of patients with solid tumors did not vary substantially with the exclusion of any individual study, which implies that the results of this meta-analysis are stable (Fig 3).

To minimize heterogeneity, the subgroup analyses were performed according to the multivariate analysis, test method, region, cancer type, and staining position. Both of the subgroup analyses with the multivariate analysis method (HR: 1.55, 95%CI: 1.09–2.21, P = 0.015) and the IHC test method (HR: 1.79, 95%CI: 1.37–2.35, P<0.001) demonstrated that decreased LKB1 expression was evidently related to poor OS in the patients with solid tumors, and the heterogeneities were similar. When stratifying by geographic region, decreased LKB1 expression was significantly associated with poor OS in patients from Asia (HR: 2.18, 95%CI: 1.66–2.86, P<0.001 with less heterogeneity), while the non-Asian subgroup exhibited no association.

Potentially relevant studies identified through database searching in Pubmed, Embase and Web of science (n=128)

Potentially relevant studies identified through database searching in CNKI (n=19)

Studies after duplicates removed (n=111)

Studies screened (n=111)

Studies excluded (n=94):
Article of review (n=7)
Laboratory research studies (n=87)

Full-text articles assessed for eligibility (n=17)

Further excluded (n=3):
The survival curve was based on LKB1 gene expression (n=2)
Specimens were metastatic tumors (n=1)

Studies included in qualitative synthesis (n=14)

Studies included in this meta-analysis (n=14)

**Fig 1. Flow diagram of the selection of eligible studies.**

doi:10.1371/journal.pone.0152674.g001

When grouped according to cancer type, the pooled HRs for lung cancer and other solid tumors were 2.16 (95%CI: 1.47–3.18, P<0.001 with more less heterogeneity) and 1.74 (95%CI: 1.23–2.45, P = 0.002), respectively. In the staining position subgroup, an intimate correlation between decreased LKB1 expression and poor OS was observed in both the cytoplasm studies (HR = 1.69, 95%CI: 1.07–2.68, P = 0.024) and another group (HR = 1.87, 95%CI: 1.30–2.68, P = 0.001), and significant heterogeneity was present (Table 3).

**Table 1. Main characteristics of the eligible studies.**

| First author | Year | Region | Type of cancer | Number of cases | Median age (range) | Adjuvant therapybefore surgery | Adjuvant therapy after surgery | Follow-up (months) | NOS score |
|---|---|---|---|---|---|---|---|---|---|
| Huang YH [13] | 2013 | China | Hepatocellular carcinoma | 70 | 57(43–72) | NR | NR | 68 | 7 |
| He TY [14] | 2014 | Taiwan | Colorectal cancer | 158 | NR | NR | NR | 81 | 5 |
| Bouchekioua-Bouzaghou K [17] | 2014 | France | Breast cancer | 154 | 57(27–87) | NR | NR | 162 | 7 |
| Shen Z [11] | 2002 | China | Breast carcinoma | 116 | 53.7(32–77) | Radiotherapy for 40 cases | Chemotherapy for 56 cases, Hormonal therapy for 43 cases | 70 | 6 |
| Tsai LH [27] | 2014 | Taiwan | Lung adenocarcinomas | 115 | NR | None | NR | 140 | 7 |
| Jiang LL [15] | 2014 | China | Non-small cell lung cancer | 142 | 58.2(31–84) | None | NR | 71 | 7 |
| Yang JY [16] | 2015 | China | Pancreatic ductaladenocarcinoma | 205 | NR | None | NR | 98 | 7 |
| Calles A [28] | 2015 | USA | Lung adenocarcinoma | 126 | 63.5(30–84) | NR | NR | 60 | 7 |
| Wang JH [26] | 2015 | China | Intrahepatic cholangiocarcinoma | 326 | NR | NR | NR | 99 | 8 |
| Lee SW [18] | 2015 | Taiwan | Hepatocellular carcinoma | 120 | NR | NR | NR | 101 | 7 |
| Morton JP [12] | 2010 | UK | Pancreatic cancer | 106 | NR | NR | NR | 95 | 6 |
| Ding XM [29] | 2005 | China | Lung adenocarcinoma | 62 | 60.5(32–77) | None | Radiotherapy/ chemotherapy | 80 | 8 |
| Yang XW [30] | 2012 | China | Gastric cancer | 100 | 65(31–85) | None | Radiotherapy/ chemotherapy | 36 | 7 |
| Huang Y [31] | 2014 | China | Gastric carcinoma | 115 | 61(37–80) | None | NR | 75 | 6 |

doi:10.1371/journal.pone.0152674.t001

## Decreased LKB1 Expression and RFS/DFS

No significant correlation between decreased LKB1 expression and RFS was observed in the patients with solid tumors in either the univariate group (HR: 1.23, 95%CI: 0.41–3.67) or the multivariate group (HR: 1.23, 95% CI: 0.35–4.33) analysis in the random-effects model with significant heterogeneity ($I^2$ = 93.70%, P<0.001; $I^2$ = 94.70%, P<0.001, respectively). Moreover, the pooled HR from the univariate analysis method with a random-effects model also indicated that no significant association existed between decreased LKB1 expression and DFS (HR: 1.42, 95% CI: 0.65–3.10) (Table 4).

## Correlations of Decreased LKB1 Expression with Clinicopath-Ological Features

The clinical and pathological parameters that were collected from the eligible studies are presented in S3 Table. Meanwhile, Table 5 summarizes the pooled results of the correlations that were identified between decreased LKB1 expression and the clinicopathological features in the patients with solid tumors. No significant correlations of decreased LKB1 expression with age, sex or tumor differentiation were observed. However, the decreased expression of LKB1 was positively associated with tumor size (OR: 1.60, 95%CI: 1.09–2.36, P = 0.017), lymph node metastasis (OR: 2.41, 95%CI: 1.53–3.78, P<0.001) and TNM stage (OR: 3.35, 95%CI: 2.20–5.09, P<0.001).

**Table 2. LKB1 evaluation and survival data of the selected studies.**

| First author | Test method | Staining position | Cut-off value | Outcome | Analysis method | HR and 95% CI |
|---|---|---|---|---|---|---|
| Huang YH [13] | IHC | Cytoplasm | Staining index scores of ≤3 | OS | UA | 3.155(1.603–6.211) |
| | | | | | MA | 2.179(1.066–4.444) |
| | | | | DFS | UA | 2.737(1.629–6.271) |
| He TY [14] | IHC | No specific description | A score equal to or lower than 100 | OS | UA | 2.364(1.576–4.112) |
| | | | | | MA | 3.146(1.876–5.276) |
| | | | | RFS | UA | 2.522(1.701–4.445) |
| | | | | | MA | 3.093(1.843–5.191) |
| Bouchekioua-Bouzaghou K [17] | IHC | Nucleus | Staining intensity recorded as 0 | OS | UA | 1.417(0.722–2.704) |
| | | | | DFS | UA | 1.279(0.732–2.225) |
| Bouchekioua-Bouzaghou K [17] | IHC | Cytoplasm | Staining intensity recorded as 0–1 | OS | UA | 0.418(0.181–0.708) |
| | | | | | MA | 0.403(0.199–0.820) |
| | | | | DFS | UA | 0.495(0.249–0.809) |
| | | | | | MA | 0.549(0.303–0.990) |
| Shen Z [11] | WB | Total protein | The bands of the breast cancer tissue in which the quantities were <0.5 | OS | UA | 3.754(1.899–10.75) |
| | | | | DFS | UA | 2.529(1.383–5.933) |
| Tsai LH [27] | IHC | No specific description | A score equal to or lower than 100 | OS | UA | 1.846(1.243–3.202) |
| | | | | | MA | 1.868(1.160–3.007) |
| | | | | RFS | UA | 1.828(1.247–3.122) |
| | | | | | MA | 1.791(1.132–2.834) |
| Jiang LL [15] | IHC | Cytoplasm | A score of 0–4 | OS | UA | 3.226(1.852–5.556) |
| | | | | | MA | 2.128(1.136–4.000) |
| Yang JY [16] | IHC | No specific description | A total score <4 | OS | UA | 2.278(1.495–3.472) |
| | | | | | MA | 1.845(1.189–2.865) |
| Calles A [28] | IHC | Cytoplasm | No staining | OS | UA | 1.44(0.92–2.28) |
| Wang JH [26] | IHC | Cytoplasm | The staining density was under the median value | OS | UA | 1.857(1.498–2.483) |
| | | | | | MA | 1.824(1.404–2.377) |

*(Continued)*

**Table 2.** (*Continued*)

| First author | Test method | Staining position | Cut-off value | Outcome | Analysis method | HR and 95% CI |
|---|---|---|---|---|---|---|
| Lee SW [18] | IHC | No specific description | The H-score was <the median | OS | UA | 0.517(0.284–0.931) |
| | | | | | MA | 0.496(0.245–1.047) |
| | | | | RFS | UA | 0.403(0.237–0.624) |
| | | | | | MA | 0.333(0.193–0.564) |
| Morton JP [12] | IHC | Cytoplasm | The histoscore was ≤100 | OS | UA | 1.877(1.280–4.318) |
| | | | | | MA | 1.87(1.09–3.22) |
| Ding XM [29] | IHC | Both nucleus and cytoplasm | The staining intensity in the neoplasms was lower than that of normal airway epithelium | OS | UA | 3.003(2.524–9.635) |
| Yang XW [30] | IHC | Both nucleus and cytoplasm | The staining intensity in the neoplasms was less than that of normal mucosa | OS | UA | 2.558(1.674–4.588) |
| Huang Y [31] | IHC | Both nucleus and cytoplasm | No staining | OS | UA | 2.514(1.026–4.092) |

doi:10.1371/journal.pone.0152674.t002

## Evaluation of Publication Bias

The shape of the funnel plot for the OS appeared to asymmetrical, indicating potential publication bias ([Fig 4](#)). However, the Begg's and Egger's tests revealed non-significant values (P = 0.322 and 0.928, respectively).

## Discussion

LKB1 is a primary upstream kinase of adenosine monophosphate-activated protein kinase (AMPK) [32] and a required element in cell metabolism for the maintenance of energy homeostasis. LKB1 exerts growth-suppressing effects by activating a group of AMPK-related kinases. The activation of AMPK-related kinases by LKB1 plays vital roles in the maintenance of cell polarity and inhibits the inappropriate expansion of cancer cells. Thus, LKB1 functions as a
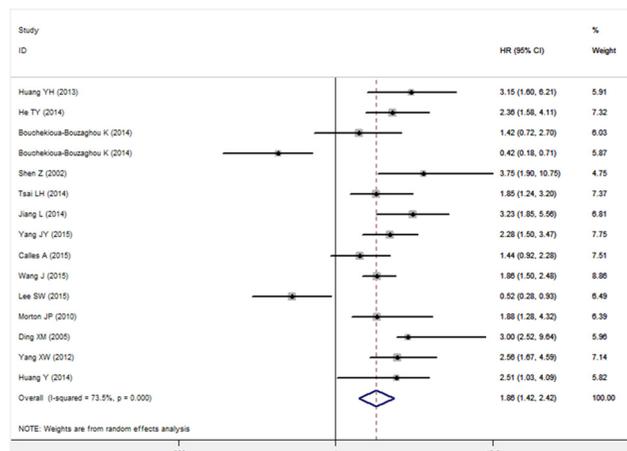


**Fig 2. Forest plot describing the association between decreased LKB1 expression and OS.**

doi:10.1371/journal.pone.0152674.g002
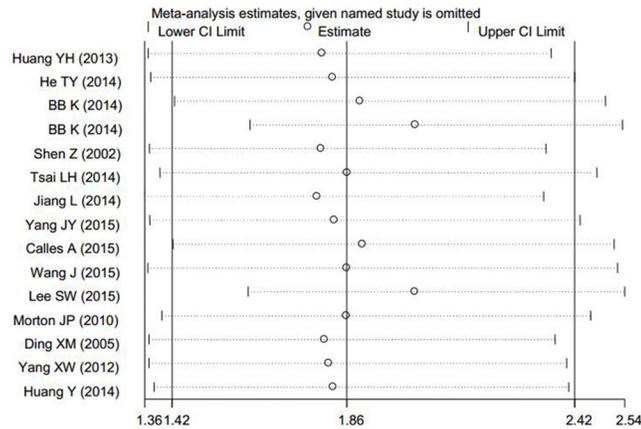
**Fig 3. Sensitivity analysis of the OS in the meta-analysis (note: BB K was used as an abbreviation for Bouchekioua-Bouzaghou K because the full name was too long and affected the typesetting of the image).**

doi:10.1371/journal.pone.0152674.g003

human tumor suppressor [33,34]. Consequently, decreases in LKB1 can promote cancer progression and are predictive of poor prognoses in patients with cancer [16,35]. However, thus far, no meta-analyses have been performed to evaluate the prognostic value of decreased LKB1 in patients with solid tumors. To the best of our knowledge, this is the first comprehensive meta-analysis of the effects of decreased LKB1 expression on the survival and clinicopathological characteristics of solid tumors.

In this meta-analysis, 14 eligible studies met the inclusion criteria. The data were organized according to OS, RFS and DFS. The combined results demonstrated that decreased LKB1 expression was associated with a poorer OS in solid tumor patients based on a random effects model. The sensitivity analysis revealed that no individual study influenced the overall results, indicating the stability of the pooled results. Additionally, no publication bias was observed. Due to significant heterogeneity between our included studies, we performed further subgroup analyses according to the analysis method, test method, region, cancer type, and staining position. With the exception of non-Asian regions, all of the subgroup analyses indicated that decreased LKB1 expression was associated with poor OS. Regarding the studies that evaluated RFS and DFS, decreased expression of LKB1 was not correlated with either of these factors. However, because the number of articles related to the analyses of RFS and DFS were both no more than 3, these results remain inconclusive and require further investigation. Furthermore,

**Table 3. Associations between decreased LKB1 expression and OS stratified according to the test method, geographic region, cancer type and staining position.**

| Categories | Subgroups | Reference number | HR (95% CI) | P-Value | Heterogeneity | |
|---|---|---|---|---|---|---|
| | | | | | I² | P-Value |
| Test method | IHC | [12–18, 26–31] | 1.79(1.37–2.35) | <0.001 | 74.2% | <0.001 |
| Region | Asian | [11, 13–16, 27, 29–31] | 2.18(1.66–2.86) | <0.001 | 67.1% | 0.001 |
| | Not Asian | [12, 17, 28] | 1.15(0.63–2.08) | 0.647 | 75.1% | 0.007 |
| Cancer type | Lung cancer | [15, 27–29] | 2.16(1.47–3.18) | <0.001 | 52.9% | 0.095 |
| | Other types | [11, 13–18, 26, 30, 31] | 1.74(1.23–2.45) | 0.002 | 78.1% | <0.001 |
| Staining position | Cytoplasm | [12, 13, 15, 17, 26, 28] | 1.69(1.07–2.68) | 0.024 | 80.4% | <0.001 |
| | The others | [11, 14, 16–18,27, 29–31] | 1.87(1.30–2.68) | 0.001 | 71.4% | 0.001 |

doi:10.1371/journal.pone.0152674.t003

**Table 4. Meta-analysis results of decreased LKB1 expression and survival.**

| Survival data | Analysis method | Reference number | HR (95% CI) | P-value | Heterogeneity | |
|---|---|---|---|---|---|---|
| | | | | | I² | P-value |
| OS | Univariate analysis | [11–18,26–31] | 1.86(1.42–2.42) | <0.001 | 73.5% | <0.001 |
| | Multivariate analysis | [12–18, 26, 27] | 1.55(1.09–2.21) | 0.015 | 76.5% | <0.001 |
| RFS | Univariate analysis | [14, 18,27] | 1.23(0.41–3.67) | 0.709 | 93.7% | <0.001 |
| | Multivariate analysis | [14, 18,27] | 1.23(0.35–4.33) | 0.746 | 94.7% | <0.001 |
| DFS | Univariate analysis | [11, 13, 17] | 1.42(0.65–3.10) | 0.376 | 83.5% | <0.001 |

doi:10.1371/journal.pone.0152674.t004

**Table 5. Meta-analysis results of the associations of decreased LKB1 expression with clinicopathological parameters.**

| Clinicopathological parameter | Reference number | Overall OR(95% CI) | P-value | Heterogeneity test(Q, I², P-value) |
|---|---|---|---|---|
| Age(≥60 vs <60) | [15, 29–31] | 0.88(0.56–1.39) | 0.583 | 1.74, 0.0%, 0.628 |
| Sex(male vs female) | [13–16, 26–30] | 0.90(0.71–1.16) | 0.418 | 5.63, 0.0%, 0.689 |
| Tumor differentiation(poor vs well) | [11, 13, 15–17, 26, 30] | 1.84(0.79–4.30) | 0.160 | 39.24, 82.2%, <0.001 |
| Tumor size(T3-4 vs T1-2) | [11, 13, 16, 17, 26, 27, 29–31] | 1.60(1.09–2.36) | 0.017 | 17.11, 47.4%, 0.047 |
| Lymph node metastasis(yes vs no) | [11, 15–17, 26, 27, 29–31] | 2.41(1.53–3.78) | <0.001 | 29.17, 69.2%, 0.001 |
| TNM stage(III-IV vs I-II) | [13–16, 26, 27, 29–31] | 3.35(2.20–5.09) | <0.001 | 18.28, 56.2%, 0.019 |

doi:10.1371/journal.pone.0152674.t005

significant associations of decreased LKB1 expression with larger tumor size, lymph node metastasis and higher TNM stage were observed. Therefore, we conclude that decreased LKB1 may serve as a biomarker for poor clinicopathological prognostic factors.

The current analyses have several important implications. First, decreased LKB1 may be a universal poor prognostic marker in solid tumors. In this meta-analysis, we included seven different cancer types, i.e., lung cancer [15,27–29], breast cancer [11,17], gastric cancer [30,31], hepatocellular cancer [13,18], pancreatic cancer [12,16], colorectal cancer [14] and intrahepatic cholangiocarcinoma [26]. The pooled results from these cancer types demonstrated that decreased LKB1 expression was associated with a poor OS and this finding can basically be extended to all solid tumors [22,36–38]. Second, we demonstrated that decreased LKB1 correlated with poor OS in the Asian region but not in the non-Asian region. This discrepancy may
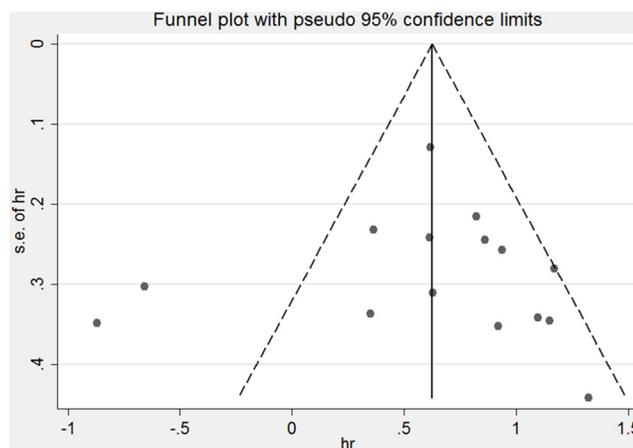


**Fig 4. Funnel plot for the assessment of potential publication bias regarding OS in the meta-analysis.**

doi:10.1371/journal.pone.0152674.g004

have been due to environmental factors that varied in the different regions and different genetic backgrounds [39,40]. Third, decreased LKB1 expression may be a reliable prognostic marker of lung cancer patients with poor OS. Our analysis results revealed that lung cancer patients with decreased expression of LKB1 exhibited significantly poorer OSs. However, because lung cancer is the leading cause of cancer-related death worldwide [41], additional original research regarding the correlation between decreased LKB1 expression and the survival data of patients with lung cancer is needed to verify our results. Fourth, different localizations and specific mutations of LKB1 may alter the association between LKB1 expression and cancer patient survival. LKB1 has different localizations in mammalian cells. The accumulation of LKB1has been detected in both the nuclei and cytoplasm of cells [42,43]. Via the formation of complexes with other proteins [43,44] and under specific conditions [45,46], LKB1 can also translocate from the nucleus to the cytoplasm. Additionally, specific mutations can lead to the loss of the ability of LKB1 to inhibit cell growth and promote cancer progression [47,48]. Thus, the possible mutations in LKB1 maybe among the reasons for the conflicting OS results that included in our meta-analysis.

However, in our meta-analysis, two studies reported inconsistent results that decreased LKB1 might correlate with a favorable survival [17,18], which showing the two obvious outliers on the left of graph in Fig 4. We suspect, aside from the possibility of different localizations and specific mutations of LKB1 discussed above, that the particular molecular phenotypes, such as methylated ERα(metERα) [17] and Skp2-dependent ubiquitination [18], as well as its related mechanisms, of the metERα/Src/PI3K complex [17] and the Skp2-mediated K63-linked polyubiquitination of LKB1 [18] may play primary roles in these contradictory phenomena.

Several limitations should be considered when interpreting our meta-analysis results. One of the main limitations is the significant heterogeneity between the included studies. However, we used a random-effects model with the pooled data. The heterogeneity among these studies could be explained by the different patient characteristics or differences in the specific study designs according to the different tumor types. Another limitation is that some of the survival data were extracted from Kaplan-Meier curves and might have introduced bias. Thus, the present statistics seem to be less reliable than those directly obtained from published studies. One additional limitation is that all of the included studies were designed as retrospective studies, and such studies are more likely to be published if they have positive results than if they have negative results. Therefore, our estimate of the association between decreased LKB1 and outcome may have been overestimated. Finally, the lack of consensus regarding the definition of the cut-off value for decreased LKB1 expression in these included studies might have led to between-study heterogeneity, and we were unable to set a baseline for decreased LKB1 expression which may have resulted in inconsistency.

In summary, this meta-analysis suggested that decreased LKB1 expression significantly contributed to poor OS in solid tumor patients. Decreased LKB1 is also a potential predictive marker for poor clinicopathological prognostic factors in patients with solid tumors. However, further studies related to specific tumor types and perspectives are required to verify the clinical utility of decreased LKB1 in solid tumors.

## Supporting Information

**S1 File. PRISMA 2009 Checklist.**
(DOC)

**S1 Table. Newcastle-Ottawa Scale (NOS) for quality assessment in meta-analysis.**
(DOCX)

**S2 Table. Quality assessment of the 14 included studies according to the NOS.**
(DOCX)

**S3 Table. Summarized data of clinical and pathological parameters from the eligible studies.**
(XLSX)

## Acknowledgments

## Author Contributions

Conceived and designed the experiments: JX QC. Performed the experiments: XC YG XXL WL. Analyzed the data: JX YZ. Contributed reagents/materials/analysis tools: YZ MXX BXH SYH SJY. Wrote the paper: JX. Contributed to the revising of the manuscript: QC.

## References

1. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nature genetics. 1998; 18(1):38–43. Epub 1998/01/13. doi: 10.1038/ng0198-38 PMID: 9425897.

2. Martin SG, Johnston D St. A role for Drosophila LKB1 in anterior-posterior axis formation and epithelial polarity. Nature. 2003; 421(6921):379–84. Epub 2003/01/24. doi: 10.1038/nature01296 PMID: 12540903.

3. Benkemoun L, Descoteaux C, Chartier NT, Pintard L, Labbe JC. PAR-4/LKB1 regulates DNA replication during asynchronous division of the early C. elegans embryo. The Journal of cell biology. 2014; 205(4):447–55. Epub 2014/05/21. doi: 10.1083/jcb.201312029 PMID: 24841566; PubMed Central PMCID: PMCPMC4033775.

4. Krawchuk D, Anani S, Honma-Yamanaka N, Polito S, Shafik M, Yamanaka Y. Loss of LKB1 leads to impaired epithelial integrity and cell extrusion in the early mouse embryo. Journal of cell science. 2015; 128(5):1011–22. Epub 2015/01/16. doi: 10.1242/jcs.162156 PMID: 25588837.

5. Mirouse V, Swick LL, Kazgan N, Johnston D St, Brenman JE. LKB1 and AMPK maintain epithelial cell polarity under energetic stress. The Journal of cell biology. 2007; 177(3):387–92. Epub 2007/05/02. doi: 10.1083/jcb.200702053 PMID: 17470638; PubMed Central PMCID: PMCPMC2064817.

6. Partanen JI, Tervonen TA, Myllynen M, Lind E, Imai M, Katajisto P, et al. Tumor suppressor function of Liver kinase B1 (Lkb1) is linked to regulation of epithelial integrity. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(7):E388–97. Epub 2012/02/07. doi: 10.1073/pnas.1120421109 PMID: 22308451; PubMed Central PMCID: PMCPMC3289385.

7. Li J, Liu J, Li P, Mao X, Li W, Yang J, et al. Loss of LKB1 disrupts breast epithelial cell polarity and promotes breast cancer metastasis and invasion. Journal of experimental & clinical cancer research: CR. 2014; 33:70. Epub 2014/09/03. doi: 10.1186/s13046-014-0070-0 PMID: 25178656; PubMed Central PMCID: PMCPMC4431490.

8. Pena CG, Nakada Y, Saatcioglu HD, Aloisio GM, Cuevas I, Zhang S, et al. LKB1 loss promotes endometrial cancer progression via CCL2-dependent macrophage recruitment. The Journal of clinical investigation. 2015. Epub 2015/09/29. doi: 10.1172/jci82152 PMID: 26413869.

9. Han X, Li F, Fang Z, Gao Y, Li F, Fang R, et al. Transdifferentiation of lung adenocarcinoma in mice with Lkb1 deficiency to squamous cell carcinoma. Nature communications. 2014; 5:3261. Epub 2014/02/18. doi: 10.1038/ncomms4261 PMID: 24531128; PubMed Central PMCID: PMCPMC3929783.

10. Marignani PA. LKB1, the multitasking tumour suppressor kinase. Journal of clinical pathology. 2005; 58(1):15–9. Epub 2004/12/30. doi: 10.1136/jcp.2003.015255 PMID: 15623475; PubMed Central PMCID: PMCPMC1770539.

11. Shen Z, Wen XF, Lan F, Shen ZZ, Shao ZM. The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2002; 8(7):2085–90. Epub 2002/07/13. PMID: 12114407.

12. Morton JP, Jamieson NB, Karim SA, Athineos D, Ridgway RA, Nixon C, et al. LKB1 haploinsufficiency cooperates with Kras to promote pancreatic cancer through suppression of p21-dependent growth

arrest. Gastroenterology. 2010; 139(2):586–97, 97 e1–6. Epub 2010/05/11. doi: 10.1053/j.gastro.2010.04.055 PMID: 20452353; PubMed Central PMCID: PMCPMC3770904.

13. Huang YH, Chen ZK, Huang KT, Li P, He B, Guo X, et al. Decreased expression of LKB1 correlates with poor prognosis in hepatocellular carcinoma patients undergoing hepatectomy. Asian Pacific journal of cancer prevention: APJCP. 2013; 14(3):1985–8. Epub 2013/05/18. PMID: 23679304.

14. He TY, Tsai LH, Huang CC, Chou MC, Lee H. LKB1 loss at transcriptional level promotes tumor malignancy and poor patient outcomes in colorectal cancer. Annals of surgical oncology. 2014; 21 Suppl 4: S703–10. Epub 2014/06/01. doi: 10.1245/s10434-014-3824-1 PMID: 24879590.

15. Jiang L, Liang X, Liu M, Wang W, Ma J, Guo Q, et al. Reduced expression of liver kinase B1 and Beclin1 is associated with the poor survival of patients with non-small cell lung cancer. Oncology reports. 2014; 32(5):1931–8. Epub 2014/09/02. doi: 10.3892/or.2014.3432 PMID: 25175672.

16. Yang JY, Jiang SH, Liu DJ, Yang XM, Huo YM, Li J, et al. Decreased LKB1 predicts poor prognosis in Pancreatic Ductal Adenocarcinoma. Scientific reports. 2015; 5:10575. Epub 2015/05/28. doi: 10.1038/srep10575 PMID: 26015068.

17. Bouchekioua-Bouzaghou K, Poulard C, Rambaud J, Lavergne E, Hussein N, Billaud M, et al. LKB1 when associated with methylatedERalpha is a marker of bad prognosis in breast cancer. International journal of cancer Journal international du cancer. 2014; 135(6):1307–18. Epub 2014/03/13. doi: 10.1002/ijc.28781 PMID: 24615515.

18. Lee SW, Li CF, Jin G, Cai Z, Han F, Chan CH, et al. Skp2-dependent ubiquitination and activation of LKB1 is essential for cancer cell survival under energy stress. Molecular cell. 2015; 57(6):1022–33. Epub 2015/03/03. doi: 10.1016/j.molcel.2015.01.015 PMID: 25728766.

19. Lin SC, Gan ZH, Yao Y, Min da L. The Prognostic Value of Forkhead Box P3 Expression in Operable Breast Cancer: A Large-Scale Meta-Analysis. PloS one. 2015; 10(8):e0136374. Epub 2015/08/26. doi: 10.1371/journal.pone.0136374 PMID: 26305693; PubMed Central PMCID: PMCPMC4549287.

20. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007; 8:16. Epub 2007/06/09. doi: 10.1186/1745-6215-8-16 PMID: 17555582; PubMed Central PMCID: PMCPMC1920534.

21. Krieg A, Riemer JC, Telan LA, Gabbert HE, Knoefel WT. CXCR4—A Prognostic and Clinicopathological Biomarker for Pancreatic Ductal Adenocarcinoma: A Meta-Analysis. PloS one. 2015; 10(6): e0130192. Epub 2015/06/20. doi: 10.1371/journal.pone.0130192 PMID: 26091099; PubMed Central PMCID: PMCPMC4474597.

22. Liu F, Qi L, Liu B, Liu J, Zhang H, Che D, et al. Fibroblast activation protein overexpression and clinical implications in solid tumors: a meta-analysis. PloS one. 2015; 10(3):e0116683. Epub 2015/03/17. doi: 10.1371/journal.pone.0116683 PMID: 25775399; PubMed Central PMCID: PMCPMC4361589.

23. Rhodes LV, Tate CR, Hoang VT, Burks HE, Gilliam D, Martin EC, et al. Regulation of triple-negative breast cancer cell metastasis by the tumor-suppressor liver kinase B1. Oncogenesis. 2015; 4:e168. Epub 2015/10/06. doi: 10.1038/oncsis.2015.27 PMID: 26436950.

24. Saigusa S, Inoue Y, Tanaka K, Toiyama Y, Kawamura M, Okugawa Y, et al. Significant correlation between LKB1 and LGR5 gene expression and the association with poor recurrence-free survival in rectal cancer after preoperative chemoradiotherapy. Journal of cancer research and clinical oncology. 2013; 139(1):131–8. Epub 2012/09/19. doi: 10.1007/s00432-012-1308-x PMID: 22986809.

25. Kline ER, Muller S, Pan L, Tighiouart M, Chen ZG, Marcus AI. Localization-specific LKB1 loss in head and neck squamous cell carcinoma metastasis. Head & neck. 2011; 33(10):1501–12. Epub 2011/09/20. doi: 10.1002/hed.21638 PMID: 21928424.

26. Wang J, Zhang K, Wang J, Wu X, Liu X, Li B, et al. Underexpression of LKB1 tumor suppressor is associated with enhanced Wnt signaling and malignant characteristics of human intrahepatic cholangiocarcinoma. Oncotarget. 2015; 6(22):18905–20. Epub 2015/06/10. PMID: 26056085.

27. Tsai LH, Chen PM, Cheng YW, Chen CY, Sheu GT, Wu TC, et al. LKB1 loss by alteration of the NKX2-1/p53 pathway promotes tumor malignancy and predicts poor survival and relapse in lung adenocarcinomas. Oncogene. 2014; 33(29):3851–60. Epub 2013/09/03. doi: 10.1038/onc.2013.353 PMID: 23995788.

28. Calles A, Sholl LM, Rodig SJ, Pelton AK, Hornick JL, Butaney M, et al. Immunohistochemical Loss of LKB1 Is a Biomarker for More Aggressive Biology in KRAS-Mutant Lung Adenocarcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2015; 21(12):2851–60. Epub 2015/03/05. doi: 10.1158/1078-0432.ccr-14-3112 PMID: 25737507.

29. Ding XM, Li ZP, Zhou PK, Duan YY, Feng HS, Xu QZ. Expression of LKB1 protein and its clinical significance in lung adenocarcinomas. Chin J Cancer Prev Treat. 2005;(17: ):10–3.

30. Yang XW, Li T, Li HS, Yang CM. Expression of LKB1 in gastric cancer and its clinical significance. Chin Arch Gen Surg (Electronic Edition). 2012;(01: ):51–6.

31. Huang Y, Xia L, Mo FG, Xu XY. Clinical significance of the expression of LKB1 and vascular endothelial growth factor in gastric cancinoma. Pract Geriatr. 2014;(09: ):734–7.

32. Hardie DG, Alessi DR. LKB1 and AMPK and the cancer-metabolism link—ten years after. BMC biology. 2013; 11:36. Epub 2013/04/17. doi: 10.1186/1741-7007-11-36 PMID: 23587167; PubMed Central PMCID: PMCPMC3626889.

33. Gan RY, Li HB. Recent progress on liver kinase B1 (LKB1): expression, regulation, downstream signaling and cancer suppressive function. International journal of molecular sciences. 2014; 15(9):16698–718. Epub 2014/09/23. doi: 10.3390/ijms150916698 PMID: 25244018; PubMed Central PMCID: PMCPMC4200829.

34. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nature reviews Cancer. 2009; 9(8):563–75. Epub 2009/07/25. doi: 10.1038/nrc2676 PMID: 19629071; PubMed Central PMCID: PMCPMC2756045.

35. Gao Y, Xiao Q, Ma H, Li L, Liu J, Feng Y, et al. LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(44):18892–7. Epub 2010/10/20. doi: 10.1073/pnas.1004952107 PMID: 20956321; PubMed Central PMCID: PMCPMC2973865.

36. Han K, Qi W, Gan Z, Shen Z, Yao Y, Min D. Prognostic value of Ezrin in solid tumors: a meta-analysis of the literature. PloS one. 2013; 8(7):e68527. Epub 2013/07/31. doi: 10.1371/journal.pone.0068527 PMID: 23894313; PubMed Central PMCID: PMCPMC3716773.

37. Wu P, Wu D, Li L, Chai Y, Huang J. PD-L1 and Survival in Solid Tumors: A Meta-Analysis. PloS one. 2015; 10(6):e0131403. Epub 2015/06/27. doi: 10.1371/journal.pone.0131403 PMID: 26114883; PubMed Central PMCID: PMCPMC4483169.

38. Zhang J, Yao YH, Li BG, Yang Q, Zhang PY, Wang HT. Prognostic value of pretreatment serum lactate dehydrogenase level in patients with solid tumors: a systematic review and meta-analysis. Scientific reports. 2015; 5:9800. Epub 2015/04/23. doi: 10.1038/srep09800 PMID: 25902419.

39. Wall JD, Yang MA, Jay F, Kim SK, Durand EY, Stevison LS, et al. Higher levels of neanderthal ancestry in East Asians than in Europeans. Genetics. 2013; 194(1):199–209. Epub 2013/02/16. doi: 10.1534/genetics.112.148213 PMID: 23410836; PubMed Central PMCID: PMCPMC3632468.

40. Qin P, Zhou Y, Lou H, Lu D, Yang X, Wang Y, et al. Quantitating and dating recent gene flow between European and East Asian populations. Scientific reports. 2015; 5:9500. Epub 2015/04/04. doi: 10.1038/srep09500 PMID: 25833680; PubMed Central PMCID: PMCPMC4382708.

41. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians. 2015; 65 (1):5–29. Epub 2015/01/07. doi: 10.3322/caac.21254 PMID: 25559415.

42. Smith DP, Spicer J, Smith A, Swift S, Ashworth A. The mouse Peutz-Jeghers syndrome gene Lkb1 encodes a nuclear protein kinase. Human molecular genetics. 1999; 8(8):1479–85. Epub 1999/07/13. PMID: 10400995.

43. Boudeau J, Baas AF, Deak M, Morrice NA, Kieloch A, Schutkowski M, et al. MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. The EMBO journal. 2003; 22(19):5102–14. Epub 2003/10/01. doi: 10.1093/emboj/cdg490 PMID: 14517248; PubMed Central PMCID: PMCPMC204473.

44. Brajenovic M, Joberty G, Kuster B, Bouwmeester T, Drewes G. Comprehensive proteomic analysis of human Par protein complexes reveals an interconnected protein network. The Journal of biological chemistry. 2004; 279(13):12804–11. Epub 2003/12/17. doi: 10.1074/jbc.M312171200 PMID: 14676191.

45. Liang HJ, Chai RC, Li X, Kong JG, Jiang JH, Ma J, et al. Astrocytic exportin-7 responds to ischemia through mediating LKB1 translocation from the nucleus to the cytoplasm. Journal of neuroscience research. 2015; 93(2):253–67. Epub 2014/09/25. doi: 10.1002/jnr.23486 PMID: 25250856.

46. Zhan YY, Chen Y, Zhang Q, Zhuang JJ, Tian M, Chen HZ, et al. The orphan nuclear receptor Nur77 regulates LKB1 localization and activates AMPK. Nature chemical biology. 2012; 8(11):897–904. Epub 2012/09/18. doi: 10.1038/nchembio.1069 PMID: 22983157.

47. Wingo SN, Gallardo TD, Akbay EA, Liang MC, Contreras CM, Boren T, et al. Somatic LKB1 mutations promote cervical cancer progression. PloS one. 2009; 4(4):e5137. Epub 2009/04/03. doi: 10.1371/journal.pone.0005137 PMID: 19340305; PubMed Central PMCID: PMCPMC2660434.

48. Qiu W, Schonleben F, Thaker HM, Goggins M, Su GH. A novel mutation of STK11/LKB1 gene leads to the loss of cell growth inhibition in head and neck squamous cell carcinoma. Oncogene. 2006; 25 (20):2937–42. Epub 2006/01/13. doi: 10.1038/sj.onc.1209325 PMID: 16407837; PubMed Central PMCID: PMCPMC1459486.