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Association between LKB1 expression and prognosis of patients with solid tumours: an updated systematic review and meta-analysis

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ABSTRACT

Objectives Liver kinase B1 (LKB1) is considered a tumour suppressor that can control cell growth and metabolism. Whether LKB1 expression levels are related to clinicopathology and prognosis is controversial. This review aimed to quantitatively examine the latest evidence on this question.

Design An updated systematic review and meta-analysis on the association between LKB1 expression and prognosis of patients with solid tumours were performed.

Data sources Eligible studies were identified through literature searches from database establishment until 15 June 2018 in the following databases: Embase, PubMed, Web of Science, Cochrane Library, China National Knowledge Infrastructure and Wan Fang databases.

Eligibility criteria The association between LKB1 expression and clinicopathological characteristics, overall survival (OS), disease-free survival (DFS) and relapse-free survival (RFS) of patients with solid tumours were reported. Sufficient data were available to calculate the OR or HR and 95% CI.

Data extraction and synthesis Relevant data were meta-analysed for OS, DFS, RFS and various clinical parameters.

Results The systematic review included 25 studies containing 6012 patients with solid tumours. Compared with patients with high LKB1 expression, patients with low expression showed significantly shorter OS in univariate analysis (HR=1.63, 95% CI 1.35 to 1.97, p<0.01) and multivariate analysis (HR=1.61, 95% CI 1.26 to 2.06, p<0.01). In contrast, the two groups showed similar DFS in univariate analysis (HR=1.49, 95% CI 0.73 to 3.01, p=0.27) as well as similar RFS in univariate analysis (HR=1.44, 95% CI 0.65 to 3.17, p=0.37) and multivariate analysis (HR=1.02, 95% CI 0.42 to 2.47, p=0.97). Patients with low LKB1 expression showed significantly worse tumour differentiation (OR=1.71, 95% CI 1.14 to 2.55, p<0.01), larger tumours (OR=1.68, 95% CI 1.24 to 2.27, p<0.01), earlier lymph node metastasis (OR=1.43, 95% CI 1.26 to 1.62, p<0.01) and more advanced tumour, node, metastases (TNM) stage (OR=1.80, 95% CI 1.56 to 2.07, p<0.01).

Conclusion Low LKB1 expression predicts shorter OS, worse tumour differentiation, larger tumours, earlier lymph node metastasis and more advanced TNM stage.

Strengths and limitations of this study

- ► This review included large sample size to reveal the relationship between the expression of liver kinase B1 (LKB1) and solid tumours.
- ► Subgroup analyses and sensitivity analyses were performed to confirm the findings.
- ► The cut-off value of LKB1 among the included studies were inconsistent.

Low LKB1 expression may be a useful biomarker of poor clinicopathology and prognosis.

INTRODUCTION

The serine/threonine kinase liver kinase B1 (LKB1), also known as STK11, was originally observed to be mutated in the genes of patients with Peutz-Jeghers syndrome.[1] LKB1 is often mutated in lung, breast, gastric and other cancers.[2–4] LKB1 plays key roles in multiple cellular processes, including cell structure control, cell cycle regulation, apoptosis and cellular metabolism.[5–7] LKB1 phosphorylates multiple substrates, including AMPK, to act as a tumour suppressor to restrict tumourigenesis and metastasis.[8] Mice with a regulatory T cell (Treg)-specific deletion of LKB1 develop a fatal inflammatory disease, and LKB1 in Treg cells acts not through signalling by AMPK or the mammalian target of rapamycin complex 1 (mTORC1) and hypoxia-inducible factor 1, but through signalling involving programmed cell death protein 1 and TNF receptor proteins.[9] LKB1 deficiency can render tumour cells sensitive to metabolic stress, which may turn out to be an antitumour strategy.[10]

Although several studies have examined the role of LKB1 in tumour inhibition, its role in the prognosis of solid tumours has not been conclusively determined. Several studies

suggest that decreased LKB1 expression indicates poor prognosis. In fact, meta-analysis showed that decreased LKB1 expression in patients with solid tumours may be related to poor prognosis and serve as a predictor of clinicopathological prognostic factors.[11] However, other studies have not reproduced these findings, and some have even suggested that decreased LKB1 may correlate with favourable survival.

Therefore, we systematically reviewed and meta-analysed the relevant literature to understand the current evidence about a relationship between LKB1 expression and prognosis in patients with solid tumours.

MATERIALS AND METHODS

Literature search strategy

The following databases were searched from database establishment to 15 June 2018 to identify studies of LKB1 expression and survival in solid tumours: PubMed, Embase, Web of Science, Cochrane Database, the Chinese National Knowledge Infrastructure and Wang Fang. Searches were carried out using terms such as LKB1, STK11, liver kinase B1, prognosis, prognostic, survival and overall survival. For example, we searched PubMed using the following strategy: (LKB1(tw) OR STK11(tw) OR 'liver kinase B1'(tw) OR 'serine-threonine kinase 11'(tw)) AND ('prognosis' (MeSH terms) OR prognoses(tw) OR prognostic(tw) OR 'prognostic factor'(tw) OR 'prognostic factors'(tw) OR factor(tw) OR factors(tw) OR outcome(tw) OR survival(tw) OR metastases(tw) OR metastasis(tw) OR migration(tw) OR transplantation(tw) OR transfer(tw) OR shift(tw) OR divert(tw) OR recurrence(tw) OR relapse(tw) OR reappear(tw) OR recur(tw) OR recidivation(tw) OR invasion(tw)).

Study inclusion and exclusion criteria

Studies were considered eligible if they met the following criteria: (1) LKB1 expression in cancer tissue (obtained via surgery or biopsy) was measured by immunohistochemistry or western blot analysis; (2) the association was studied between LKB1 expression and clinicopathological characteristics, overall survival (OS), disease-free survival (DFS) or recurrence-free survival (RFS) of patients with solid tumours; (3) sufficient data were published for calculating an OR or HR and 95% CI and (4) the study was published as a full-text article in English or Chinese. If we retrieved multiple studies conducted by the same research group and involving overlapping patient populations, only the most recent or most complete study was included in the meta-analysis. Articles were excluded if they (1) were duplicate publications; (2) were case reports, reviews, letters or animal studies or (3) did not report survival outcomes.

Study quality assessment

Two reviewers independently assessed the quality of included studies using the standard Newcastle-Ottawa Scale (NOS) from 0 to 9. NOS scores of 9–7 were defined as high quality, 6–4 as intermediate quality and 3–1 as low quality.

Data extraction

Two researchers (YHR and FJZ) independently screened all titles and abstracts identified in the initial search. Articles remaining after this screen were read in full and assessed for eligibility. The following types of data were extracted: (1) name of first author, publication year, country, type of cancer and number of patients; (2) patient's age, gender, follow-up time, type of LKB1 assay, intracellular location where LKB1 staining was examined, LKB1 cut-off value for classifying expression as high or low, survival data (OS, DFS, RFS), statistical method used to analyse survival data; (3) tumour differentiation, tumour size, lymph node metastasis and tumour stage. All data were cross-checked by two researchers, and disagreements were resolved by a third reviewer (XMY). If study information was incomplete or unclear, we contacted the corresponding author in an attempt to collect accurate information.

Statistical analysis

Correlation between LKB1 expression and OS of patients with solid tumours was evaluated in terms of HR and 95% CI. If a study showed Kaplan-Meier survival curves but not HRs with 95% CI, data were extracted from survival curves using Engauge Digitizer 4.1 and the Tierney's table. Correlation between LKB1 expression and clinicopathological characteristics of patients with solid tumours was evaluated in terms of OR and 95% CI.

HRs and ORs were meta-analysed using the random-effects model in R software. P values were two-sided and values <0.05 were considered to be statistically significant.

I^2 was used to assess statistical heterogeneity. If I^2 was >50%, heterogeneity was considered to exist among all included studies, and we conducted a subgroup analysis to investigate its possible source. If I^2 was <50%, heterogeneity among all included studies was regarded as insignificant, and data were directly pooled.

To assess the stability of our meta-analysis results, we conducted a sensitivity analysis to test the influences of individual studies on the pooled HR or p value for the remaining studies. Potential for publication bias was assessed by examining funnel plots, Begg's test and Egger's test of survival data.

RESULTS

A total of 4858 potentially relevant studies were identified in literature searches, of which 3374 were excluded as duplicate publications. After screening titles and abstracts, 50 studies were read in full, leading to 25 that were included in the meta-analysis[12–36] (figure 1). Data from all 25 studies were meta-analysed to examine the potential correlation of LKB1 expression with clinicopathological characteristics. Data from 24 studies were meta-analysed to examine the potential correlation

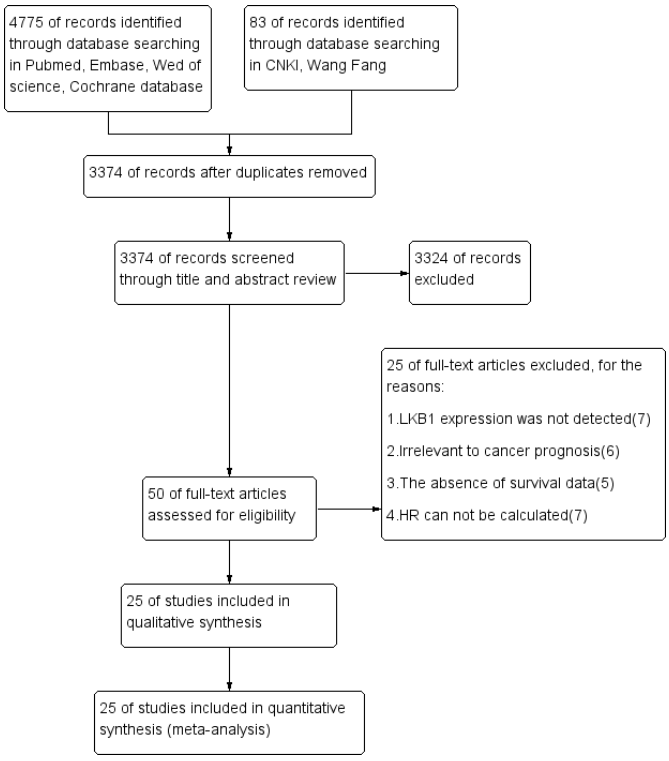

Figure 1 Flow diagram of the eligible studies. CNKI, Chinese National Knowledge Infrastructure; LKB1, liver kinase B1.

between LKB1 expression and OS. Data from five studies were used to analyse the potential correlation between LKB1 expression and DFS. Four studies reported the association of LKB1 expression with RFS.

Description of studies

The 25 studies in the systematic review involved 6012 patients from six countries: China, the USA, France, the UK, Canada and Egypt. Data on OS were reported in 24 studies, data on RFS in 5 studies and data on DFS in 4 studies. Patients covered a range of cancers, including cancers of the lung, breast, prostate or pancreas; gastric cancer; hepatocellular carcinoma; oesophagus squamous cancer; colorectal cancer; glioma and laryngeal squamous cell carcinoma. Tables 1–2 summarise the characteristics of the included studies. Online supplementary table 1 lists clinicopathological characteristics and LKB1 expression. Eight studies had a NOS score of 8; 11 studies, 7; 6 studies, 6 and 3 studies, 5 (online supplementary table 2 and online supplementary table 3).

Of the 25 studies, 16 reported HRs from multivariate analysis, which we used directly. For the nine remaining studies, we estimated HRs for OS, DFS and RFS from survival curves and Tierney's table.

Association between LKB1 expression and OS

Given heterogeneity among the studies (I^2=74.0%, p<0.001), a random-effects model was used to meta-analyse the data. The pooled HR describing OS for patients with low LKB1 expression relative to OS for patients with high expression is shown in figure 2. Decreased LKB1 expression was significantly associated with OS: low expression was associated with significantly higher risk of poor survival (HR=1.63, 95% CI 1.35 to 1.97, p<0.01).

To assess the predictive role of decreased LKB1, subgroup analysis was performed after stratifying the results based on multivariate analysis, type of LKB1 assay, country, cancer type and intracellular location of LKB1 staining that was examined. Subgroup analysis based on multivariate analysis showed that decreased LKB1 expression was related to poor OS in table 3 (HR=1.61, 95% CI 1.26 to 2.06, p<0.001 with significant heterogeneity). This relationship was observed for the following cancer types: lung cancer (HR=2.07, 95% CI 1.60 to 2.69, p<0.01, I^2=0%), pancreatic cancer (HR=2.16, 95% CI 1.53 to 3.05, p<0.001, I^2=0%), gastric cancer (HR=2.11, 95% CI 1.60 to 3.01, p<0.01, I^2=0%) and breast cancer (HR=1.26, 95% CI 1.15 to 1.37, p<0.01). However, this relationship was not observed in the case of hepatocellular carcinoma (HR=1.27, 95% CI 0.84 to 1.94, p=0.26 with significant heterogeneity).

Among Asian patients, decreased LKB1 expression was associated with significantly shorter OS (HR=1.70, 95% CI 1.42 to 2.05, p<0.01); this relationship was not observed among non-Asian patients (HR=1.15, 95% CI 0.63 to 2.08, p=0.65) (table 3).

Pooled HR for the subgroup of patients tested by anti-LKB1 immunohistochemistry was 1.58 (95% CI 1.33 to 1.88, p<0.01). Low LKB1 expression based on cytoplasmic staining predicted significant adverse prognosis (HR=1.78, 95% CI 1.49 to 2.13, p<0.01). This relationship was not observed when the judgement of low LKB1 expression was based on nuclear staining (HR=1.25, 95% CI 0.85 to 1.85, p=0.26, I^2=0%) (table 3).

Details of the subgroup analysis are listed in table 3. The results of the sensitivity analysis showed that the exclusion of each single study did not alter the results significantly (figure 3). These results suggest that our meta-analysis gave credible results.

Association of LKB1 expression with DFS and RFS

Studies showed significant heterogeneity, so data were meta-analysed using a random-effects model. Low LKB1 expression did not show a significant association with RFS based on univariate analysis (HR=1.44, 95% CI 0.65 to 3.17, p=0.37) or multivariate analysis (HR=1.02, 95% CI 0.42 to 2.47, p=0.97). Similarly, no significant correlation was observed between LKB1 expression and DFS based on univariate analysis and random-effects meta-analysis (HR=1.49, 95% CI 0.73 to 3.01, p=0.27) (table 4).

Association between LKB1 expression and clinicopathological characteristics

Meta-analysis of the relationship between LKB1 expression and clinicopathological characteristics (table 5) failed to show a significant association of decreased LKB1 expression with age (OR=0.78, 95% CI 0.57 to 1.05, p=0.10) or sex (OR=0.97, 95% CI 0.78 to 1.19, p=0.76).

Table 1 Main characteristics of included studies and Newcastle-Ottawa Scale (NOS) scores

Study	Year	Country	Type of cancer	No. of cases		Age (years), median (range)	Follow-up, mo.	NOS score
				Low LKB1	High LKB1			
Ding XM[12]	2005	China	Lung adenocarcinoma	24	38	60.5 (32–77)	80	7
Tsai LH[13]	2013	China	Lung adenocarcinoma	44	71	NR	140	7
Jiang LL *et al*[14]	2014	China	Non-small cell lung cancer	33	109	58.2 (31–84)	71	7
Calles A *et al*[15]	2015	USA	Lung adenocarcinoma	42	84	63.5 (30–84)	60	7
Shen Z *et al*[16]	2002	China	Breast carcinoma	38	83	53.7 (32–77)	70	6
Bouchekioua-Bouzaghou K *et al*[17]	2014	France	Breast cancer	94	60	56.87 (27–87)	162	7
Bouchekioua-Bouzaghou K *et al*[17]	2014	France	Breast cancer	102	52	56.5 (27–87)	162	
Chen IC *et al*[18]	2016	China	Breast cancer	161	408	48	120	6
Chen IC *et al*[18]	2016	China	Breast cancer	88	189	54	120	
Chen IC *et al*[18]	2016	UK and Canada	Breast cancer	494	494	61.3	300	5
Chen IC *et al*[18]	2016	UK and Canada	Breast cancer	488	487	62.6	300	
HamdyA. Azim *et al*[19]	2016	Egypt	Breast cancer	12	20	51.3 (25–82)	82.8	6
HamdyA. Azim *et al*[19]	2016	Egypt	Breast cancer	11	21	51.3 (25–82)	82.8	
Morton JP *et al*[20]	2010	UK	Pancreatic cancer	20	86	NR	95	7
Yang JY *et al*[21]	2015	China	Pancreatic ductal adenocarcinoma	36	169	NR	97	8
Li DZ *et al*[23]	2018	China	Pancreatic neuroendocrine tumour	38	33	NR	190	8
Yang XW *et al*[22]	2012	China	Gastric cancer	76	24	65 (31–85)	38	7
Huang Y *et al*[24]	2014	China	Gastric carcinoma	24	91	61 (37–80)	75	6
Ma LG *et al*[25]	2016	China	Gastric cancer	62	47	57 (31–84)	99	8
Sun JJ *et al*[36]	2016	China	Gastric cancer	107	48	NR	70	6
Yin M *et al*[26]	2017	China	Gastric cancer	78	32	62 (23–79)	72	7
Huang YH *et al*[27]	2013	China	Hepatocellular carcinoma	31	39	57 (43–72)	68	7
Lee SW *et al*[28]	2015	China	Hepatocellular carcinoma	13	27	NR	101	7
Wu CC *et al*[29]	2018	China	Hepatocellular carcinoma	41	52	NR	54	7
Wang JH[30]	2015	China	Intrahepatic cholangiocarcinoma	187	129	NR	99	8
Ma JJ	2014	China	Oesophagus squamous cancer	73	47	NR	60	8
He TY *et al*[32]	2014	China	Colorectal cancer	63	95	NR	80.5	5
Lu JL *et al*[33]	2015	China	Prostate cancer	78	31	NR	60	7
Huang JH *et al*[27]	2017	China	Glioma	92	88	50.8 (10–86)	118	8
He SS *et al*[35]	2017	China	Laryngeal squamous cell carcinoma	128	80	NR	212.2	8

LKB1, liver kinase B1; NR, no resources.

Table 2 LKB1 expression levels and survival

Study	Assay method	Staining location	Cut-off value	Outcome	Analysis method	HR and 95% CI
Ding XM[12]	IHC	Both nucleus and cytoplasm	Lower than in normal airway epithelium	OS	UA	3.003 (1.524 to 5.865)
Tsai LH *et al*[13]	IHC	No specific description	Score ≤100	OS	UA	1.846 (1.147 to 2.952)
					MA	1.868 (1.160 to 3.007)
				RFS	UA	1.828 (1.247 to 3.122)
					MA	1.791 (1.132 to 2.834)
Jiang LL *et al*[14]	IHC	Cytoplasm	Score 0–4	OS	UA	3.226 (1.856 to 5.586)
					MA	2.128 (1.136 to 4.000)
Calles A *et al*[15]	IHC	Cytoplasm	No staining	OS	UA	1.440 (0.910 to 2.270)
ShenZ *et al*[16]	WB	Total protein	Bands of the breast cancer tissue in which the quantities were <0.5	OS	UA	3.754 (1.583 to 8.932)
				DFS	UA	2.529 (1.383 to 5.933)
Bouchekioua-Bouzaghou K *et al*[17]	IHC	Cytoplasm	Staining intensity recorded as 0–1	OS	UA	0.418 (0.211 to 0.828)
					MA	0.403 (0.199 to 0.820)
				DFS	UA	0.495 (0.249 to 0.809)
					MA	0.549 (0.303 to 0.990)
Bouchekioua-Bouzaghou K *et al*[17]	IHC	Nucleus	Staining intensity recorded as 0	OS	UA	1.417 (0.722 to 2.734)
				DFS	UA	1.278 (0.732 to 2.225)
Chen IC *et al*[18]	IHC	No specific description	Score 0 or 1	OS	UA	1.200 (0.670 to 2.150)
					MA	0.766 (0.453 to 1.296)
Chen IC *et al*[18]	IHC	No specific description	Score 0 or 1	OS	UA	0.980 (0.600 to 1.610)
					MA	1.054 (0.665 to 1.671)
Chen IC *et al*[18]	Microarray data	No specific description	Lower than the median expression level	OS	UA	1.600 (1.360 to 1.894)
					MA	0.937 (0.772 to 1.138)
Chen IC *et al*[18]	Microarray data	No specific description	Lower than the median expression level	OS	UA	1.090 (0.910 to 1.300)
					MA	1.024 (0.839 to 1.250)
HamdyA. Azim *et al*[19]	IHC	Cytoplasm	Staining intensity recorded as 0	RFS	UA	1.110 (0.160 to 7.490)
					MA	0.810 (0.220 to 3.030)
HamdyA. Azim *et al*[19]	IHC	Nucleus	Staining intensity recorded as 0	RFS	UA	5.220 (0.23 to 118.460)
					MA	0.360 (0.150 to 0.100)
Morton JP *et al*[20]	IHC	Cytoplasm	Histoscore ≤100	OS	UA	1.877 (1.020 to 3.448)
					MA	1.870 (1.090 to 3.220)
Yang JY *et al*[21]	IHC	No specific description	Total score <4	OS	UA	2.278 (1.495 to 3.472)
					MA	1.845 (1.189 to 2.856)
Li DZ *et al*[23]	IHC	Cytoplasm	Strong immunostaining in ≤50% of the cells and/or weak staining	OS	UA	5.310 (0.200 to 142.482)
				DFS	UA	2.190 (0.410 to 11.700)

Continued

Table 2 Continued

Study	Assay method	Staining location	Cut-off value	Outcome	Analysis method	HR and 95% CI
Yang XW[22]	IHC	Both nucleus and cytoplasm	Staining intensity in the neoplasm less than that in normal mucosa	OS	UA	2.558 (1.554 to 4.233)
Huang Y *et al*[24]	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	2.514 (1.260 to 5.022)
Ma LG *et al*[25]	IHC	Both nucleus and cytoplasm	Scores ≤1	OS	UA	2.310 (1.250 to 4.270)
					MA	3.527 (1.491 to 10.630)
Sun JJ *et al*[36]	IHC	Both nucleus and cytoplasm	Scores of 0 and 1+ indicate negative result	OS	UA	1.450 (0.540 to 3.900)
					MA	4.431 (1.363 to 14.407)
Yin M *et al*[26]	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	1.070 (0.460 to 2.470)
Huang YH *et al*[27]	IHC	Cytoplasm	Staining index score ≤3	OS	UA	3.155 (1.603 to 6.211)
					MA	2.179 (1.066 to 4.44)
				DFS	UA	2.737 (1.629 to 6.271)
Lee SW *et al*[28]	IHC	Both nucleus and cytoplasm	H-score was lower than the median	OS	UA	0.517 (0.284 to 0.931)
					MA	0.333 (0.193 to 0.564)
Wu CC *et al*[29]	IHC	No specific description	Histoscore ≤150	OS	UA	3.130 (0.910 to 10.840)
					MA	4.260 (1.870 to 9.690)
				RFS	UA	2.020 (0.870 to 4.720)
					MA	2.050 (1.110 to 3.810)
Wang JH[30]	IHC	Cytoplasm	Staining density lower than the median value	OS	UA	1.857 (1.438 to 2.386)
					MA	1.824 (1.404 to 2.377)
Ma JJ *et al*[25]	IHC	Both nucleus and cytoplasm	Score 0–4	OS	UA	0.570 (0.330 to 0.980)
He TY *et al*[32]	IHC	No specific description	Score ≤100	OS	UA	2.364 (1.466 to 3.812)
					MA	3.146 (1.876 to 5.276)
				RFS	UA	2.522 (1.701 to 4.445)
					MA	3.093 (1.843 to 5.191)
Lu JL *et al*[33]	IHC	No specific description	Staining of fewer than 20% of the tissue cells or no staining	OS	UA	6.310 (0.420 to 94.730)
					MA	3.981 (1.698 to 9.336)
Huang JH *et al*[34]	IHC	No specific description	Percentage of positive cells ≤35% and/or staining intensity score 0–1	OS	UA	3.350 (1.490 to 7.510)
					MA	3.022 (1.002 to 6.016)
He SS *et al*[35]	IHC	Nucleus	Score ≤4	OS	UA	1.170 (0.720 to 1.900)
					MA	1.628 (1.060 to 2.500)

DFS, disease-free survival; IHC, immunohistochemistry; LKB1, liver kinase B1; MA, multivariate analysis; OS, overall survival; RFS, relapse-free survival; UA, univariate analysis; WB,western blot.

In contrast, low LKB1 expression was significantly related to worse differentiation (OR=1.17, 95% CI 1.14 to 2.55, p<0.01), deeper invasion (OR=1.68, 95% CI 1.24 to 2.27, p<0.01), earlier lymph node metastasis (OR=1.43, 95% CI 1.26 to 1.62, p<0.01) and more advanced clinical stage (OR=1.80, 95% CI 1.56 to 2.07, p<0.01).

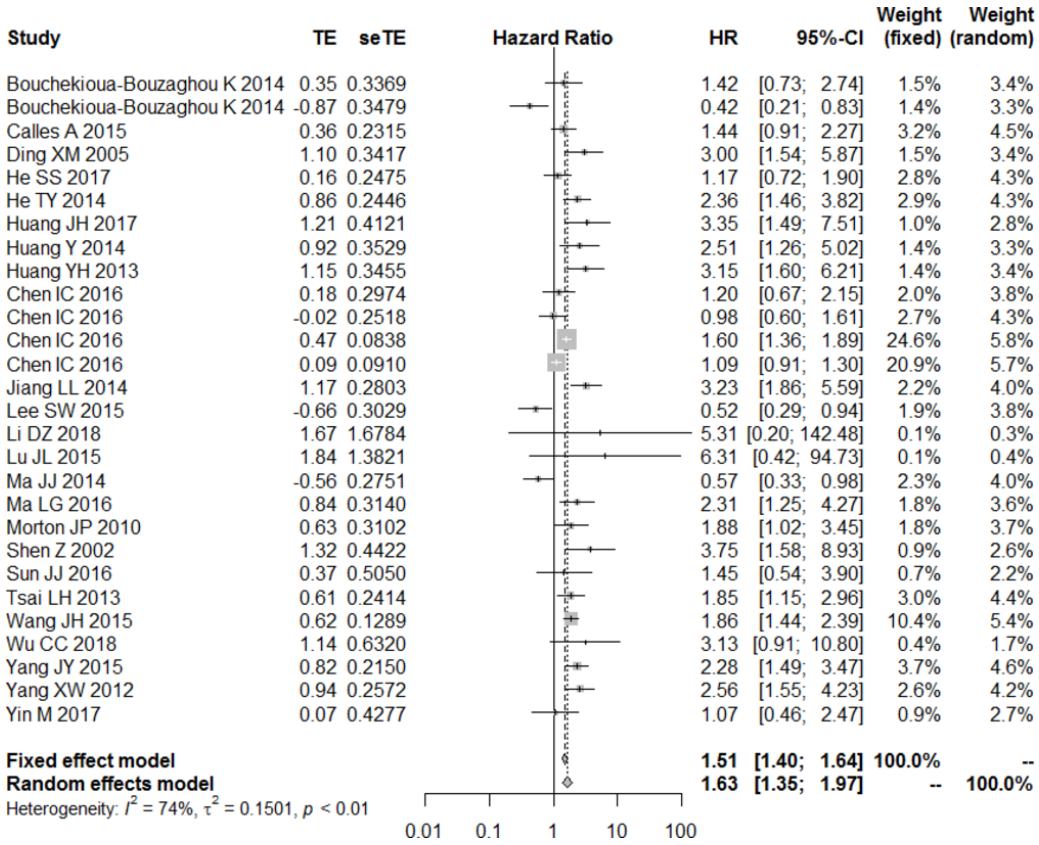

Figure 2 Forest plot of the association between decrease liver kinase B1 expression and overall survival.

Table 3 Subgroup analyses of the association between LKB1 expression and OS after stratification by statistical analysis method, LKB1 assay method, region, cancer type and intracellular staining location

				Heterogeneity	
Stratification criterion	**Value**	**HR (95% CI)**	**P value**	I^2	**P value**
Analysis method	Univariate	1.63 (1.35 to 1.97)	<0.001	74%	<0.001
	Multivariate	1.61 (1.26 to 2.06)	<0.001	81%	<0.001
Assay method	IHC	1.58 (1.33 to 1.88)	<0.001	76%	<0.001
Region	Asian	1.70 (1.42 to 2.05)	<0.001	77%	<0.001
	Not Asian	1.15 (0.63 to 2.08)	0.65	75%	0.007
Cancer type	Lung	2.07 (1.60 to 2.69)	<0.001	53%	0.09
	Breast	1.26 (1.15 to 1.37)	<0.001	79%	<0.001
	Gastric	2.11 (1.60 to 3.01)	<0.001	0%	0.41
	Pancreatic	2.16 (1.53 to 3.05)	<0.001	0%	0.76
	Hepatocellular carcinoma	1.27 (0.84 to 1.94)	0.26	89%	<0.001
	Others	1.63 (1.35 to 1.96)	<0.001	79%	<0.001
Staining position	Both nucleus and cytoplasm	1.50 (1.31 to 1.17)	<0.001	80%	<0.001
	Cytoplasm	1.78 (1.49 to 2.13)	<0.001	77%	<0.001
	Nucleus	1.25 (0.85 to 1.85)	0.26	0%	0.65
	Others	1.36 (1.25 to 1.47)	<0.001	75%	<0.001
NOS scores	High quality	1.53 (1.19 to 1.96)	<0.001	77%	<0.001
	Intermediate quality	1.79 (1.36 to 1.92)	<0.001	75%	<0.001

IHC, immunohistochemistry; LKB1, liver kinase B1; NOS, Newcastle-Ottawa Scale; OS, overall survival; RFS, relapse-free survival.

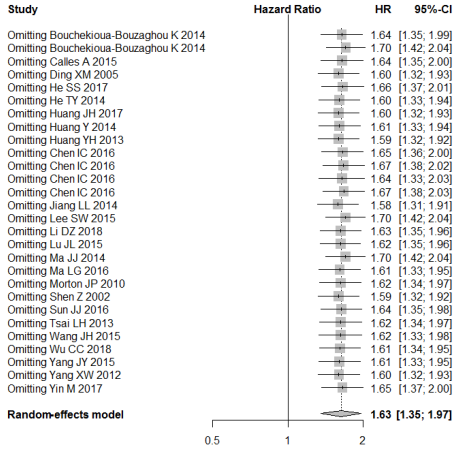

Figure 3 Sensitivity analysis of overall survival in the meta-analysis.

Results are shown as individual and pooled OR with 95% CIs.

Publication bias

Funnel plots of OS appeared asymmetric (figure 4), suggesting the possibility of publication bias among the included studies. However, findings with Begg's test (p=0.5402) and Egger's test (p=0.2414) implied no publication bias.

DISCUSSION

This meta-analysis suggests that among patients with many kinds of solid tumours, low LKB1 expression is associated with worse OS, whereas LKB1 expression does not appear to significantly influence DFS or RFS. This suggests that low LKB1 expression may be a predictor of unfavourable prognosis. In fact, the available evidence suggests an association of low LKB1 expression with worse tumour differentiation, deeper invasion, more advanced clinical stages and earlier metastasis to lymph nodes and other organs. These findings are consistent with previous conclusions,[11] and they were confirmed in our data set using sensitivity analysis.

Some potentially interesting findings emerged from subgroup analyses conducted after stratifying the data according to various criteria. Our meta-analysis linked low LKB1 expression with poor prognosis in Asians but not in non-Asians, which may reflect genetic and environmental differences. While low LKB1 expression was associated with worse prognosis in patients with certain types of cancer (lung, gastric, pancreatic, breast), this was not the case in patients with hepatocellular carcinoma. This difference may relate to different comorbidities associated with the types of cancer. Lung cancer, stomach cancer, breast cancer and pancreatic cancer have high incidence rates around the world, and more studies have been done. The association between low expression of LKB1 and poor prognosis was observed when low expression was based on cytoplasmic staining, but not when it was based on nuclear staining. The reason may be that the regulation of mTORC1 by LKB1 and AMPK occurs on the exterior of RAB7/LAMP1-positive lysosomal membranes.[37] In this regulation, LKB1 phosphorylates and activates cell energy-sensing AMPK, which in turn negatively affects TORC1, which is important for controlling energy metabolism, cell survival and cell growth under conditions of metabolic stress, such as nutrient deficiency. Further studies are needed to elucidate the mechanism of action of LKB1.

Our meta-analysis suggests that at least in many types of solid tumours, LKB1 acts as a tumour suppressor. This is consistent with several studies in the literature. For example, a decrease in LKB1 expression as a result of HBx-mediated p53 inactivation may be responsible for colony formation and invasiveness in hepatocellular carcinoma.[29] LKB1 deficiency in some tumours may be associated with upregulation of glutamate dehydrogenase 1, which activates CamKK2 and its downstream effector AMPK to increase metastatic potential.[38] LKB1 loss may drive ovarian serous tumourigenesis by disrupting apical-basal polarity in the presence of mutated p53 in fallopian tube cells.[39] On the one hand, several studies have suggested an oncogenic role for LKB1 and AMPK under certain conditions,[40] such as when class III phosphatidylinositol-3-OH kinase is inactivated.[41] Further work is needed to clarify under what conditions LKB1 acts as a tumourigenic or tumour-suppressing molecule.

Table 4 Meta-analysis results of decreased LKB1 expression and patient's prognosis

Prognosis	Analysis method	HR (95% CI)	P value	Heterogeneity	
				I^2	P value
OS	Univariate analysis	1.63 (1.35 to 1.97)	<0.01	74.0%	<0.001
	Multivariate analysis	1.61 (1.26 to 2.06)	<0.001	81.0%	<0.001
RFS	Univariate analysis	1.44 (0.65 to 3.17)	0.37	85%	<0.001
	Multivariate analysis	1.02 (0.42 to 2.47)	0.97	95%	<0.001
DFS	Univariate analysis	1.49 (0.73 to 3.01)	0.27	78%	0.001

DFS, disease-free survival; LKB1, liver kinase B1; OS, overall survival; RFS, relapse-free survival.

Table 5 Meta-analysis of the association of decreased LKB1 expression with clinicopathological characteristics

	OR (95% CI)	P value	Heterogeneity		
			Q test	I^2	P value
Age (≥60, <60 years)	0.78 (0.57 to 1.05)	0.10	4.04	0%	0.78
Sex (male, female)	0.97 (0.78 to 1.19)	0.76	9.06	0%	0.77
Tumour differentiation (poor, well)	1.71 (1.14 to 2.55)	<0.01	59.5	75%	<0.001
Tumour size (T3–T4, T1–T2)	1.68 (1.24 to 2.27)	<0.01	43.34	61%	<0.001
Lymph node metastasis (yes, no)	1.43 (1.26 to 1.62)	<0.01	58.41	74%	<0.001
TNM stage (III–IV, I–II)	1.80 (1.56 to 2.07)	<0.01	88.8	81%	<0.001

LKB1, liver kinase B1; TNM, tumour, node, metastases.

The results of our meta-analysis should be interpreted with caution given several limitations. First, we had to assess OS, DFS and/or RFS from Kaplan-Meier survival curves in several studies, such that HRs and 95% CIs were estimated indirectly. Second, studies showed substantial heterogeneity for outcomes, although we did attempt to minimise the effects of such heterogeneity by using a random-effects meta-analysis model, performing subgroup analyses and checking results through sensitivity analysis. Third, there is no consensus on LKB1 cut-off values for defining expression as low or high, which may influence conclusions about correlations and their clinical significance. Fourth, the funnel plots suggest the potential for publication bias. This may reflect the generally observed bias towards publication of positive findings. Fifth, our meta-analysis did not account for numerous other factors that may also affect prognosis, such as comorbidities and treatment history. In most cases, this information was not reported in the included studies.

Our results justify the design of rigorous in vitro and animal studies designed to explore how LKB1 influences the prognosis of various types of solid cancers. Ultimately, this work should be extended through human studies, preferentially randomised controlled trials.

CONCLUSIONS

The available evidence links low LKB1 expression with poor prognosis in patients with various types of solid tumours. This suggests that LKB1 may be a biomarker for various cancers. These findings should be verified and extended in human studies, and the mechanisms underlying the association of LKB1 expression and prognosis should be explored.

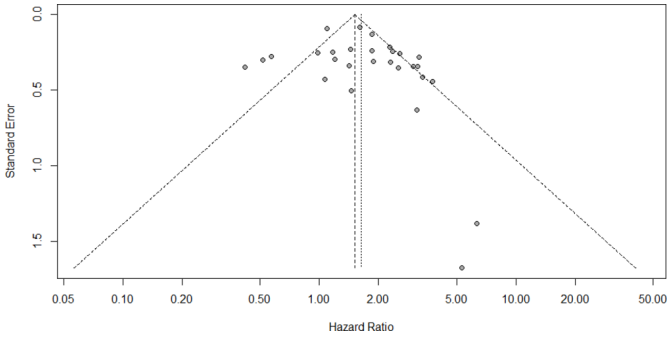

Figure 4 Funnel plot for the potential publication bias.

Contributors XMY and HYM designed the study. FJZ, HYM and RRJ conducted systematic search, searched literature and extracted data. YHR analysed the data. YHR and FJZ wrote the first draft of the article. JT, XHZ, JLW, QQL and RRH contributed significant knowledge content and critical expertise and revisions to the manuscript.

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