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

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Abstract

Objectives. Liver kinase B1 (LKB1) is considered a tumor 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.

Methods. Eligible studies were identified through a literature search up to June 15, 2018 in the following databases: Embase, PubMed, Web of Science, China National Knowledge Infrastructure and Wan Fang. Relevant data were meta-analyzed for overall survival (OS), disease-free survival (DFS), recurrence-free survival (RFS) and various clinical parameters.

Results. The systematic review included 25 studies containing 6,012 patients with solid tumors. Compared to patients with high LKB1 expression, patients with low expression showed significantly shorter OS in univariate analysis (HR1.61, 95%CI 1.36-1.92, P<0.01) and multivariate analysis (HR1.61, 95%CI 1.26-2.06, P<0.01). In contrast, the two groups showed similar DFS in univariate analysis (HR1.49, 95%CI 0.73-3.01, P=0.27) as well as similar RFS in univariate analysis (HR1.44, 95%CI 0.65-3.17, P=0.37) and multivariate analysis (HR1.02, 95%CI 0.42-2.47, P=0.97). Patients with low LKB1 expression showed significantly worse tumor differentiation (OR1.71, 95%CI 1.14-2.55, P<0.01), larger tumors (OR1.68, 95%CI 1.24-2.27, P<0.01), earlier lymph node metastasis (OR 1.43, 95%CI 1.26-1.62, P<0.01) and more advanced TNM stage (OR 1.80, 95%CI 1.56-2.07, P<0.01).

Conclusion. Low LKB1 expression predicts shorter OS, worse tumor differentiation, larger tumors, earlier lymph node metastasis and more advanced TNM stage. Low LKB1 expression may be a useful biomarker of poor clinicopathology and prognosis.

Strengths and limitations of this study. (1) Meta-analysis of 25 studies involving 6,012 patients in six countries found the evidence of a relationship between LKB1 expression and solid tumor prognosis and clinicopathology.

(2) 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.

(3) Results interpretation should pay attention to the study of high heterogeneity.

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 of tenmutated in lung, breast, gastric and other cancers [2-4]. LKB1 plays 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 tumor suppressor to restrict tumorigenesis and metastasis[8]. Mice with a 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 complex1 (mTORC1) and Hif-1, but through signalling involving pd-1 and TNF receptor proteins[9]. LKB1 deficiency can render tumor cells sensitive to metabolic stress, which may turn out to be an anti-tumor strategy[10].

Although several studies have examined the role of LKB1 in tumor inhibition, its role in the prognosis of solid tumors 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 tumors 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 favorable survival.

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

Materials and Methods

Literature search strategy

The following databases were searched through June 15, 2018 to identify studies of LKB1 expression and survival in solid tumors: PubMed, Embase, Web of Science, 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) LKB1expression in cancer tissue (obtained via surgery or biopsy) was measured by immunohistochemistry or Western blotting; (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 tumors; (3)sufficient data were published for calculating an odds ratio (OR) or hazard ratio (HR) and 95% confidence interval (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 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 analyze survival data; (3) tumor differentiation, tumor size, lymph node metastasis and TNM stage. All data were cross-checked by two researchers, and disagreements were resolved by a third reviewer (JHZ). 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 tumors 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 (sourceforge.net/projects/digitizer) and the Tierney table

(www.biomedcentral.com/content/supplementary/1745-6215-8-16S1.xls).Correlation

between LKB1 expression and clinicopathological characteristics of patients with solid tumors was evaluated in terms of OR and 95%CI.

HRs and ORs were meta-analyzed using the random-effects model in Review Manager 5.3 (Cochrane Collaboration, Copenhagen, Denmark). P values were two-sided and values <0.05 were considered statistically significant.

I^2 was used to assess statistical heterogeneity. If I^2>50%, heterogeneity was considered to exist among all included studies, and we conducted a subgroup analysis to investigate its possible source. If I^2< 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 by excluding individual studies one at a time and recalculating the pooled HR or P value for the remaining studies. Potential for publication bias was assessed by examining funnel plots of survival data.

Results

A total of 4,838 potentially relevant studies were identified in literature searches, of which 3,374 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] (Fig.1). Data from all 25

studies were meta-analyzed to examine the potential correlation of LKB1 expression with clinicopathological characteristics. Data from 24 studies were meta-analyzed to examine the potential correlation between LKB1 expression and OS. Data from only one study were used to analyze the potential correlation between LKB1 expression and RFS.

Description of studies

The 25 studies in the systematic review involved 6,012 patients from six countries: China, USA, France, UK, Canada, and Egypt. Data on OS were reported in 24 studies, data on RFS in five studies, and data on DFS in four studies. Patients covered a range of cancers, including cancers of the lung, breast, prostate or pancreas; gastric cancer; hepatocellular carcinoma; esophagus squamous cancer; colorectal cancer; glioma; and laryngeal squamous cell carcinoma. Tables 1-2 summarize the characteristics of the included studies. Table 3 lists clinicopathological characteristics and LKB1 expression. Eight studies had an NOS score of 8; 11studies, 7; 6 studies, 6; and 3 studies, 5 (Table1).

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=76.0%, P<0.001), a random-effects model was used to meta-analyze the data. The pooled HR describing OS for patients with low LKB1 expression relative to OS for patients with high expression is shown in Fig.2A. Decreased LKB1 expression was significantly associated with OS: low expression was associated with significantly higher risk of poor survival (HR1.61, 95%CI 1.36-1.92, 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 (HR 1.61, 95%CI 1.26–2.06, P <0.001; Fig.2B). This relationship was observed for the following cancer types: lung cancer (HR 2.07, 95%CI 1.60-2.69, P<0.01), pancreatic cancer (HR 2.16, 95%CI 1.53-3.05, P<0.001), gastric cancer (HR 2.19, 95%CI 1.60-3.01, P<0.01), and breast cancer (HR1.26, 95%CI 1.15-1.37, P<0.01). However, this relationship was not observed in the case of hepatocellular carcinoma (HR1.27, 95%CI 0.84-1.94, P=0.26).

Among Asian patients, decreased LKB1 expression was associated with significantly shorter OS (HR1.71, 95%CI 1.42-2.07, P<0.01); this relationship was not observed among non-Asian

patients (HR1.15, 95%CI 0.63-2.08, P=0.65).

Pooled HR for the subgroup of patients tested by anti-LKB1 immunohistochemistry was 1.58 (95%CI 1.33–1.89, P<0.01). Low LKB1 expression based on cytoplasmic staining predicted significant adverse prognosis (HR1.78, 95%CI 1.49-2.13, P<0.01). This relationship was not observed when the judgment of low LKB1 expression was based on nuclear staining (HR1.25, 95%CI 0.85-1.85, P=0.26).

Details of the subgroup analysis are listed in Table 4.The results of the sensitivity analysis showed that the exclusion of each single study did not alter the results significantly (data not shown). 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-analyzed using a random-effect model. Low LKB1 expression did not show a significant association with RFS based on univariate analysis (HR 1.44, 95%CI 0.65-3.17, P=0.37) or multivariate analysis (HR 1.02, 95%CI 0.42-2.47, P=0.97; Fig.2C). Similarly, no significant correlation was observed between LKB1 expression and DFS based on univariate analysis and random-effect meta-analysis (HR

1.49, 95%CI 0.73-3.01, P=0.27; Fig. 2D).

Association between LKB1 expression and clinicopathological characteristics

Meta-analysis of the relationship between LKB1 expression and clinicopathological characteristics (Fig.3) failed to show a significant association of decreased LKB1 expression with age (OR 0.78, 95%CI 0.57-1.05, P=0.10) or sex (OR 0.97, 95%CI 0.78-1.19, P=0.76). In contrast, low LKB1 expression was significantly related to worse differentiation (OR 1.17, 95%CI 1.14-2.55, P<0.01), deeper invasion (OR 1.68, 95%CI 1.24-2.27, P<0.01), earlier lymph node metastasis (OR 1.43, 95%CI 1.26-1.62, P<0.01), and more advanced clinical stage (OR 1.80, 95%CI 1.56-2.07, P<0.01).

Results are shown as individual and pooled OR with 95% confidence intervals

Publication bias

Funnel plots of OS appeared asymmetric (Fig.4), suggesting the possibility of publication bias among the included studies.

Discussion

This meta-analysis suggests that among patients with many kinds of solid tumors, 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 unfavorable prognosis. In fact, the available evidence suggests an association of low LKB1 expression with worse tumor differentiation, deeper invasion, more advanced clinical stage, 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 co-morbidities 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 tumors, LKB1 acts as a tumor 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 [41]. LKB1 deficiency in some tumors may be associated with up-regulation of glutamatede

hydrogenase 1, which activates CamKK2 and its downstream effector AMPK to increase metastatic potential [42]. LKB1 loss may drive ovarian serous tumorigenesis 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 forLKB1 and AMPK under certain conditions[38], such as when class III phosphatidylinositol-3-OH kinase is inactivated[40]. Further work is needed to clarify under what conditions LKB1 acts as a tumorigenic or tumor-suppressing molecule.

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 minimize the effects of such

heterogeneity by using a random-effect 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 toward publication of positive findings. Fifth, our meta-analysis did not account for numerous other factors that may also affect prognosis, such as co-morbidities 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 randomized controlled trials.

Conclusions

The available evidence links low LKB1 expression with poor prognosis in patients with various types of solid tumors. 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.

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Contributors YXM and MHY designed the study. ZFJ, MHY and JRR conduct systematic search, search literature and extract data. RYH analyzed the data. RYH and ZFJ wrote the first draft of the article. TJ, ZXH, WJL and HRR contributed significant knowledge content and critical expertise and revisions to the manuscript.

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Table1. Main characteristics of included studies and Newcastle-Ottawa scale scores.

Study	Year	Country	Type of cancer	No. cases		Age in yr, median(range)	Follow-up, mo.	NOS score
				Low LKB1	High LKB1			
Ding XM	2005	China	Lung adenocarcinoma	24	38	60.5(32–77)	80	7
Tsai LH	2013	China	Lung adenocarcinomas	44	71	NR	140	7
Jiang LL	2014	China	Non-small cell lung cancer	33	109	58.2(31-84)	71	7
Calles A	2015	USA	Lung adenocarcinoma	42	84	63.5(30-84)	60	7
Shen Z	2002	China	Breast carcinoma	38	83	53.7(32-77)	70	6
Bouchekioua-Bouzaghou K	2014	France	Breast cancer	94	60	56.87(27-87)	162	7
Bouchekioua-Bouzaghou K	2014	France	Breast cancer	102	52	56.5 (27-87）	162	
Chen IC	2016	China	Breast cancer	161	408	48	120	6
Chen IC	2016	China	Breast cancer	88	189	54	120	
Chen IC	2016	UK and	Breast cancer	494	494	61.3	300	5

		Canada						
Chen IC	2016	UK and Canada	Breast cancer	488	487	62.6	300	
HamdyA.Azim	2016	Egypt	Breast Cancer	12	20	51.3(25-82)	82.8	6
HamdyA.Azim	2016	Egypt	Breast Cancer	11	21	51.3(25-82)	82.8	
Morton JP	2010	UK	Pancreatic cancer	20	86	NR	95	7
Yang JY	2015	China	Pancreatic ductal adenocarcinoma	36	169	NR	97	8
Li DZ	2018	China	Pancreatic neuroendocrine tumor	38	33	NR	190	8
Yang XW	2012	China	Gastric Cancer	76	24	65(31-85)	38	7
Huang Y	2014	China	Gastric carcinoma	24	91	61(37-80)	75	6
Ma LG	2016	China	Gastric Cancer	62	47	57(31-84)	99	8
Sun JJ	2016	China	Gastric Cancer	107	48	NR	70	6
Yin M	2017	China	Gastric Cancer	78	32	62(23-79)	72	7
Huang YH	2013	China	Hepatocellular carcinoma	31	39	57(43-72)	68	7
Lee SW	2015	China	Hepatocellular carcinoma	13	27	NR	101	7
Wu CC	2018	China	Hepatocellular carcinoma	41	52	NR	54	7

Wang JH	2015	China	Intrahepatic cholangiocarcinoma	187	129	NR	99	8
Ma JJ	2014	China	Esophagus squamous cancer	73	47	NR	60	8
He TY	2014	China	Colorectal cancer	63	95	NR	80.5	5
Lu JL	2015	China	Prostate Cancer	78	31	NR	60	7
Huang JH	2017	China	Glioma	92	88	50.8(10-86)	118	8
He SS	2017	China	Laryngeal squamous cell carcinoma	128	80	NR	212.2	8

Table 2. LKB1 expression levels and survival.

Study	Assay method	Staining location	Cut-off value	Outcome	Analysis method	HR and 95%CI
Ding XM	IHC	Both nucleus and cytoplasm	Lower than in normal airway epithelium	OS	UA	3.003(2.524-9.635)
Tsai LH	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	IHC	Cytoplasm	Score of 0-4	OS	UA	3.226(1.852-5.556)
					MA	2.128(1.136–4.000)
Calles A	IHC	Cytoplasm	No staining	OS	UA	1.44(0.92–2.28)
ZhenZ	WB	Total protein	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)
Bouchekioua-Bouzaghou K	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)
Bouchekioua-	IHC	nucleus	Staining intensity recorded as 0	OS	UA	1.417(0.722-2.704)

Bouzaghou K				DFS	UA	1.279(0.732-2.225)
Chen IC	IHC	No specific description	A score of 0 or 1	OS	UA	1.2(0.67-2.15)
					MA	0.766(0.453–1.296)
Chen IC	IHC	No specific description	A score of 0 or 1	OS	UA	0.98(0.6-1.61)
					MA	1.054(0.665-1.671)
Chen IC	microarray data	No specific description	Lower than the median expression level	OS	UA	1.6(0.9-1.25)
					MA	0.937(0.772–1.138)
Chen IC	microarray data	No specific description	Lower than the median expression level	OS	UA	1.09(0.91-1.3)
					MA	1.024(0.839–1.250)
HamdyA.Azim	IHC	Cytoplasm	Staining intensity recorded as 0	RFS	UA	1.11(0.16-7.49)
					MA	0.81(0.22-3.03)
HamdyA.Azim	IHC	Nucleus	Staining intensity recorded as 0	RFS	UA	5.22(0.23-118.46)
					MA	0.36(0.15-0.10)
Morton JP	IHC	Cytoplasm	Histoscore was⩽100	OS	UA	1.877(1.280–4.318)
					MA	1.87(1.09–3.22)
Yang JY	IHC	No specific description	A total score<4	OS	UA	2.278(1.495–3.472)
					MA	1.845(1.189–2.865)
Li DZ	IHC	Cytoplasm	Strong immunostaining in ⩽ 50%	OS	UA	5.31(0.2-144.02)

			of the cells and/or weak staining			
				DFS	UA	2.19(0.41-11.7)
Yang XW	IHC	Both nucleus and cytoplasm	Staining intensity in the neoplasmless than that innormal mucosa	OS	UA	2.558(1.674-4.588)
Huang Y	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	2.514(1.026–4.092)
Ma LG	IHC	Both nucleus and cytoplasm	Scores ⩽1	OS	UA	2.31(1.25-4.28)
					MA	3.527(1.491-10.630)
Sun JJ	IHC	Both nucleus and cytoplasm	Scores of 0 and 1+ indicate negative result	OS	UA	1.45(0.54-3.91)
					MA	4.431 (1.363-14.407)
Yin M	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	1.07 (0.46-2.46）
Huang YH	IHC	Cytoplasm	Staining index score⩽ 3	OS	UA	3.155(1.603-6.211)
					MA	2.179(1.066-4.444)
				DFS	UA	2.737(1.629-6.271)
Lee SW	IHC	Both nucleus and cytoplasm	H-score was lower than the median	OS	UA	0.517(0.284–0.931)
					MA	0.333(0.193–0.564)
Wu CC	IHC	No specific description	Histoscore was⩽150	OS	UA	3.13(0.91-10.84)

					MA	4.26(1.87-9.69)
				RFS	UA	2.02(0.87-4.72)
					MA	2.05(1.11-3.81)
Wang JH	IHC	Cytoplasm	Staining density lower than the median value	OS	UA	1.857(1.498–2.483)
					MA	1.824(1.404–2.377)
Ma JJ	IHC	Both nucleus and cytoplasm	Score of 0–4	OS	UA	0.57(0.33-0.97)
He TY	IHC	No specific description	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)
Lu JL	IHC	No specific description	Staining of fewer than 20% of the tissue cells or no staining	OS	UA	6.31(0.42-94.67)
					MA	3.981 (1.698–9.336)
Huang JH	IHC	No specific description	Percentage of positive cells ⩽ 35%and/or staining intensity score of 0-1.	OS	UA	2.02(1.07-3.83)
					MA	3.022(1.002-6.016)
He SS	IHC	Nucleus	Score ⩽4	OS	UA	1.17(0.72-1.9)
					MA	1.628(1.060–2.500)

Table 3. LKB1 expression and clinicopathological characteristics.

Study	LKB1 expression	Age		Sex		Tumor differentiation		Tumor size		Lymph node metastasis		TNM stage	
		⩾60	<60	Male	Female	Poor	Well	T3-	T1-	Yes	No	Ⅲ-Ⅳ	Ⅰ-Ⅱ

								T4	**T2**				
Huang YH	Low			26	5	23	8	15	16			19	12
	High			31	8	17	22	20	19			27	12
He TY	Low												
	High												
Bouchekioua-Bouzaghou K	Low (cytoplasmic staining)					69	25	26	68	50	44		
	High					54	6	18	42	38	22		
	Low(nuclear staining)					83	19	34	68	63	39		
	High					40	12	10	42	25	27		
ShenZ	Low					35	3	13	25				
	High					69	14	11	73				
Tsai LH	Low			25	19			9	35	35	9	28	16
	High			41	30			9	62	31	40	24	47
Jiang LL	Low	16	17	17	16	23	10			18	15	12	21
	High	49	60	65	44	34	75			44	65	23	86
Yang JY	Low			16	20	32	4	35	1	17	19	16	20
	High			101	68	159	10	132	37	45	124	31	138
Calles A	Low			14	28								
	High			25	59								
Wang JH	Low			122	65	162	25	112	100	76	111	117	70

	High			93	46	90	49	63	51	42	97	56	83
Morton JP	Low												
	High												
Ding XM	Low	12	12	13	11			9	15	3	21	22	2
	High	21	17	14	24			7	31	8	20	15	23
Yang XW	Low	52	24	60	16	62	14	57	19	59	17	48	19
	High	16	8	20	4	20	4	13	11	7	17	6	11
Wu CC	Low	17	24	32	9							26	15
	High	25	27	45	7							32	20
Yin M	Low	43	35	54	24	57	21	71	7	56	22	68	20
	High	19	13	23	9	12	20	24	8	11	21	12	20
Huang Y	Low	51	17					65	26	64	27	80	11
	High	40	7					16	8	8	16	10	14
Ma LG	Low	51	22	60	13	60	13	48	25	24	49	31	42
	high	36	11	36	11	24	23	24	23	5	42	6	41
Chen IC	low					126	25	16	145	67	91	23	138
	High					311	81	34	372	152	253	57	351
	Low					83	2	6	82	56	32	36	52
	High					177	12	15	174	106	83	66	123
	Low					457	37	20	474	241	253	85	369
	High					459	35	26	468	235	259	88	406
	Low					392	49	29	451	241	238	88	393
	High					398	46	24	446	213	260	85	388

Li DZ	Low			26	12	6	32						
	High			16	17	1	32						
Huang YH	Low			56	36			52	40			77	15
	High			54	34			20	68			43	55
HamdyA. Azim	Low												
	High												
Lu JL	Low											47	31
	High											11	20
He SS	Low												
	High												
Ma JJ	Low	29	33	40	22	27	35	44	18	51	11	28	34
	High	30	17	31	16	19	28	18	29	29	18	9	38
Lee SW	Low												
	High												
Sun JJ	Low	60	47	79	28	78	29	73	34	60	47	55	52
	High	28	20	42	6	38	10	22	26	16	32	8	40

Table 4. Subgroup analyses of the association between LKB1 expression and OS after stratification by statistical analysis method,LKB1 assay method,country, cancer type, and intracellular staining location.

Stratification criterion	Value	HR(95%CI)	P value	Heterogeneity	
				I^2	P value
Analysis method	Univariate	1.61(1.36-1.92)	<0.001	76%	<0.001
	Multivariate	1.61(1.26-2.06)	<0.001	81%	<0.001
Assay method	IHC	1.58(1.33-1.89)	<0.001	77%	<0.001
	The others				
Country	Asian	1.71(1.42-2.07)	<0.001	78%	<0.001
	Not Asian	1.15(0.63-2.08)	0.65	75%	0.007
Cancer type	Lung	2.07(1.60-2.69)	<0.001	53%	0.09
	Breast	1.26(1.15-1.37)	<0.001	79%	<0.001
	Gastric	2.19(1.60-3.01)	<0.001	10%	0.34
	Pancreatic	2.16(1.53-3.05)	<0.001	0%	0.76
	Hepatocellular carcinoma	1.27(0.84-1.94)	0.26	89%	<0.001
	The others	1.63(1.35-1.96)	<0.001	79%	<0.001
Staining position	Both nucleus and cytoplasm	1.69(1.33-2.16)	<0.001	76%	<0.001
	Cytoplasm	1.78(1.49-2.13)	<0.001	77%	<0.001

	Nucleus	1.25(0.85-1.85)	0.26	0%	0.65
	Other	1.36(1.25-1.47)	<0.001	75%	<0.001

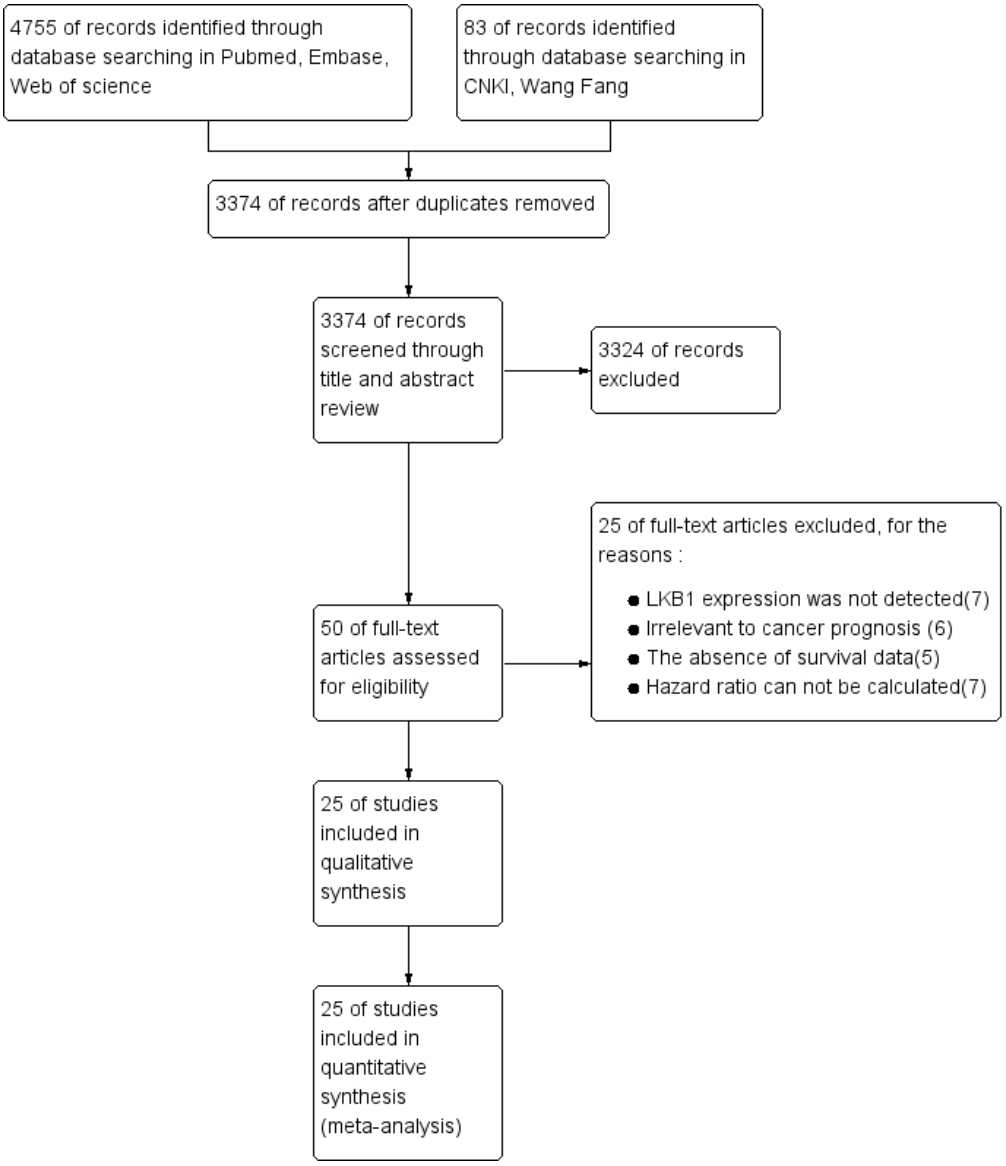

Figure 1.Flow diagram of study inclusion and meta-analysis.

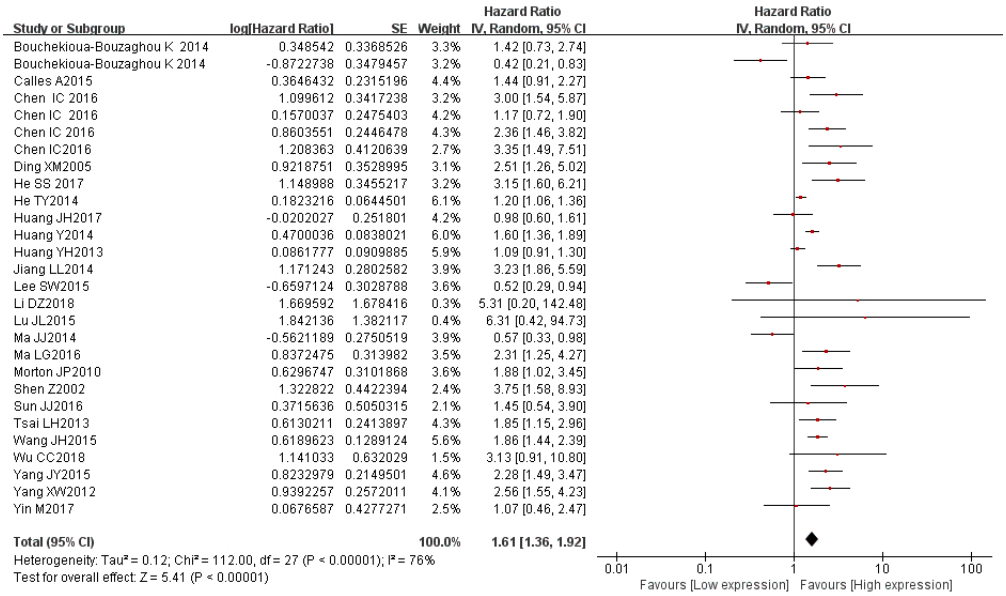

Figure 2A.Forest plot of OS by univariate analysis.

330x197mm (72 x 72 DPI)

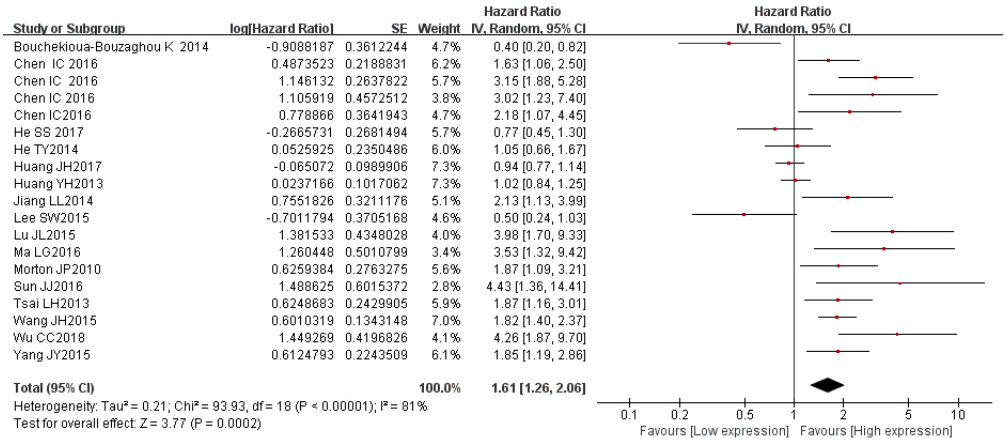

Figure 2B. Forest plot of OS by multivariate analysis.

330x146mm (72 x 72 DPI)

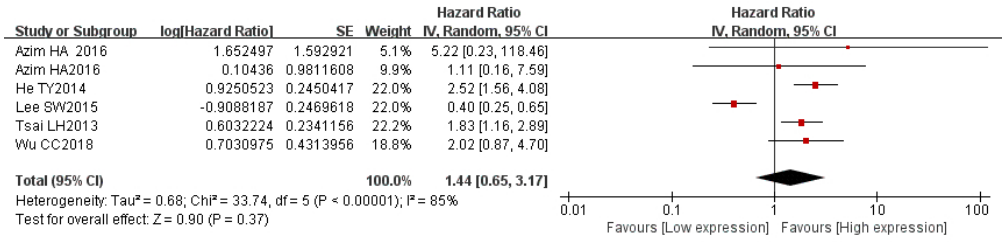

Figure 2C. Forest plot of RFS by univariate analysis.

302x73mm (72 x 72 DPI)

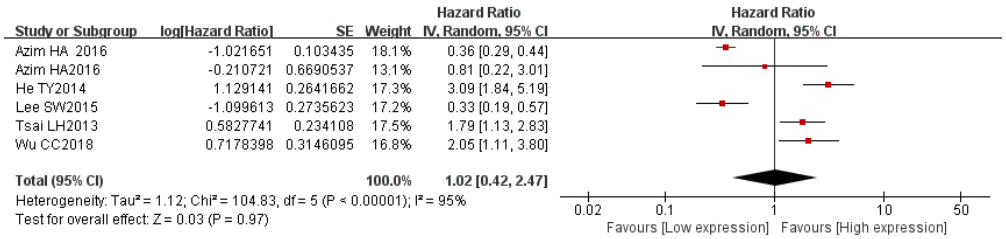

Figure 2D.Forest plot of RFS by multivariate analysis.

302x73mm (72 x 72 DPI)

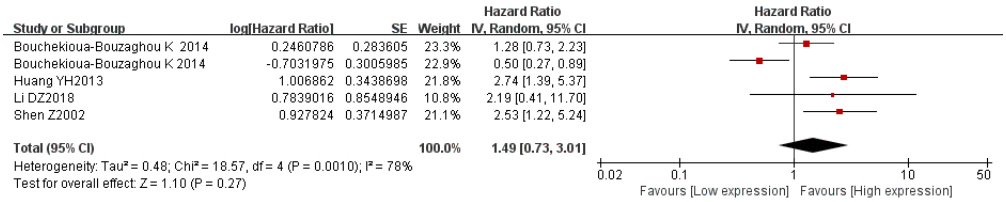

Figure 2E.Forest plot of DFS by univariate analysis.

330x67mm (72 x 72 DPI)

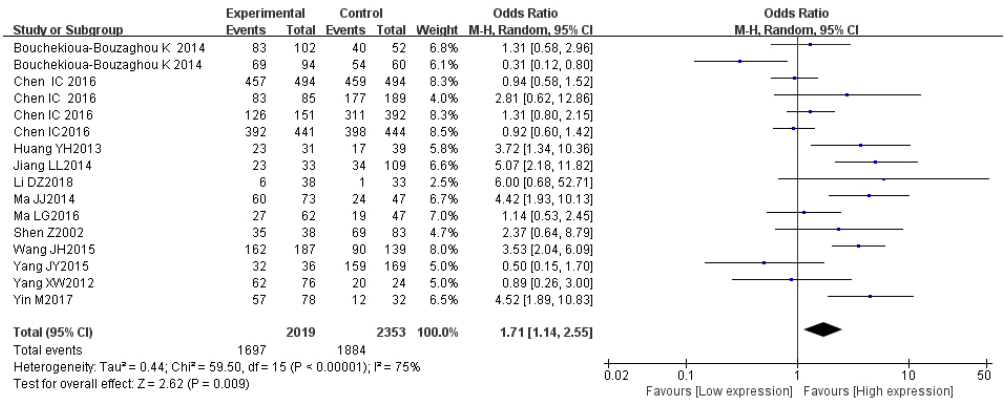

Figure 3A.Meta-analysis of the relationship between low LKB1 expression and tumor differentiation.

335x135mm (72 x 72 DPI)

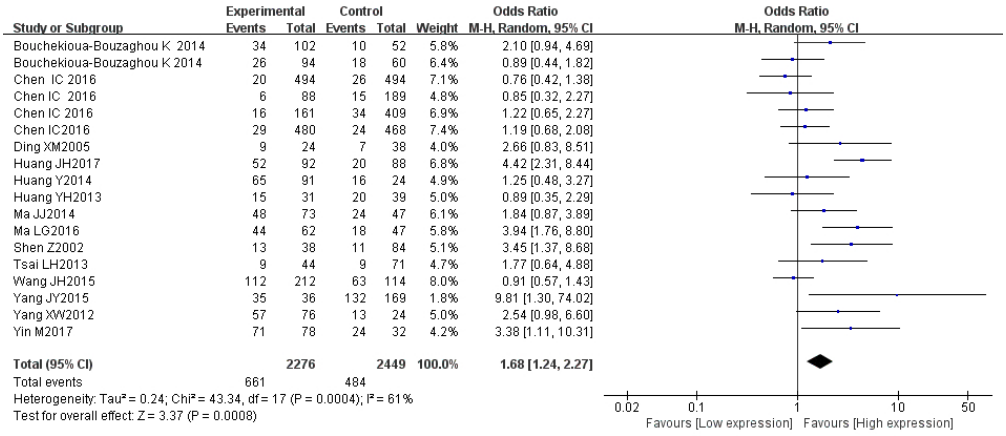

Figure3B.Meta-analysis of the relationship between low LKB1 expression and tumor size.

335x146mm (72 x 72 DPI)

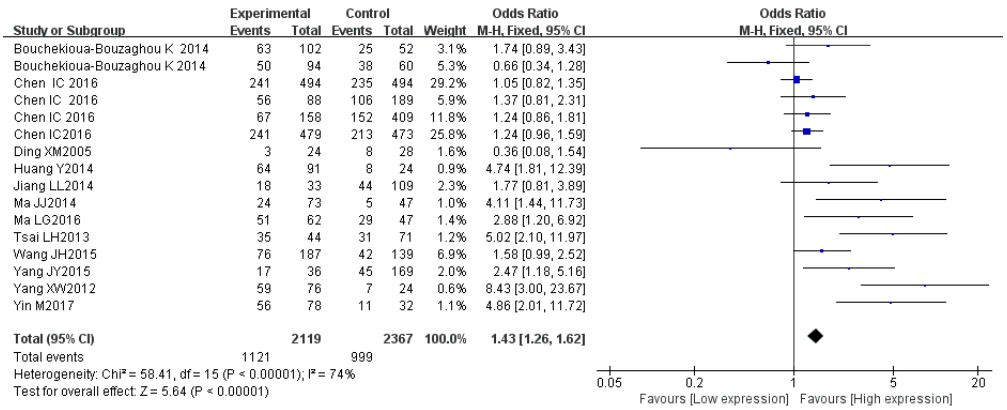

Figure3C.Meta-analysis of the relationship between low LKB1 expression and lymph node metastasis.

329x135mm (72 x 72 DPI)

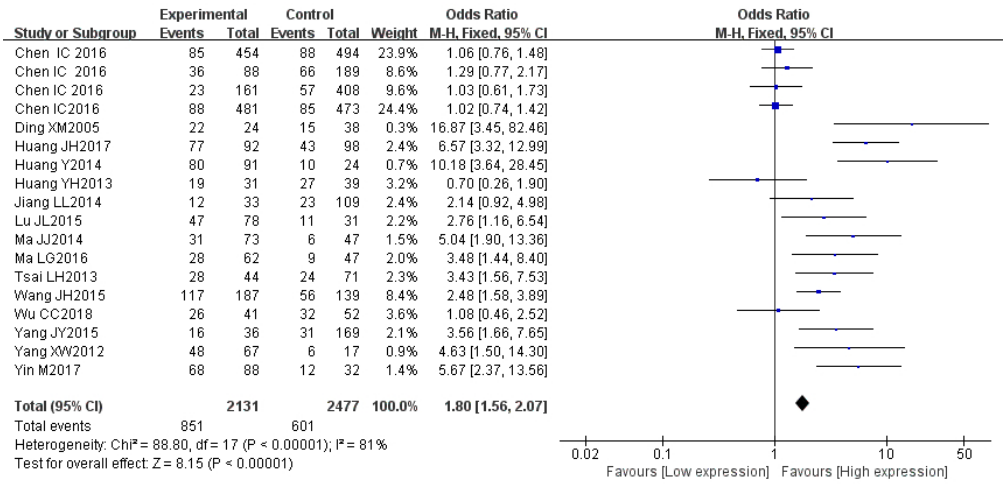

Figure3D.Meta-analysis of the relationship between low LKB1 expression and TNM stage.

302x146mm (72 x 72 DPI)

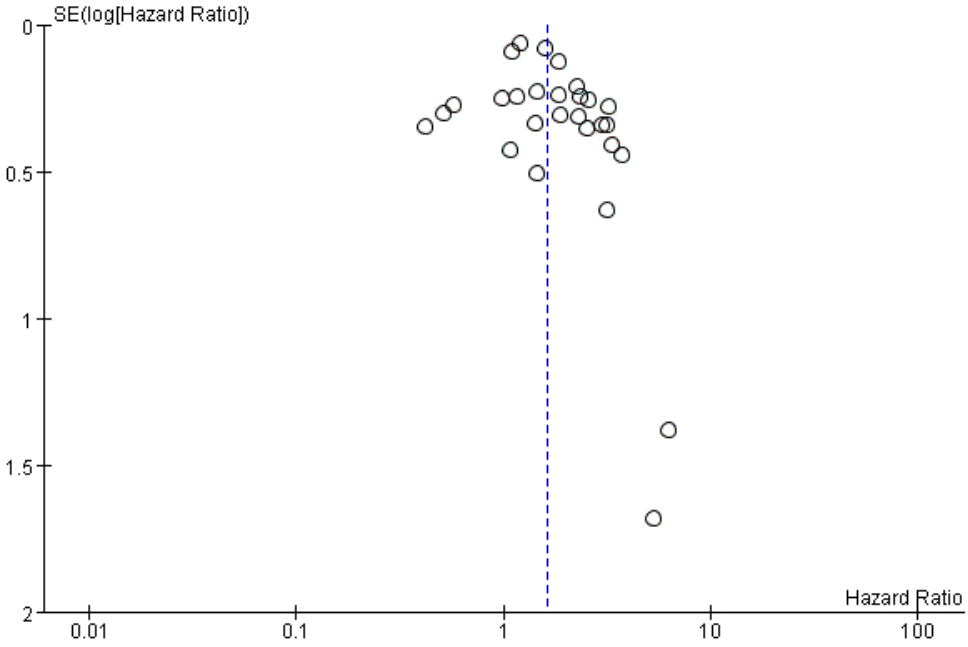

Figure4. Funnel plot of OS by univariate analysis.

211x141mm (72 x 72 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a meta-analysis.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

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

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Diagnostics
Keywords:	LKB1, STK11, liver kinase B1, prognosis

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

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Strengths and limitations of this study

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

Abstract

Objectives. Liver kinase B1 (LKB1) is considered a tumor 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 tumors were performed.

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

Eligibity 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 tumors were reported. Sufficient data was available to calculate the odds ratio (OR) or hazard ratio (HR) and 95% confidence interval (CI).

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

Results. The systematic review included 25 studies containing 6,012 patients with solid tumors. Compared to patients with high LKB1 expression, patients with low expression showed significantly shorter OS in univariate analysis (HR =1.63, 95%CI 1.35-1.97, $P < 0.01$) and multivariate analysis (HR = 1.61, 95%CI 1.26-2.06, $P < 0.01$). In contrast, the two groups showed similar DFS in univariate analysis (HR = 1.49, 95%CI 0.73-3.01, $P = 0.27$) as well as similar RFS in univariate analysis (HR = 1.44, 95%CI 0.65-3.17, $P = 0.37$) and

multivariate analysis (HR = 1.02, 95%CI 0.42-2.47, P = 0.97). Patients with low LKB1 expression showed significantly worse tumor differentiation (OR = 1.71, 95%CI 1.14-2.55, P < 0.01), larger tumors (OR = 1.68, 95%CI 1.24-2.27, P < 0.01), earlier lymph node metastasis (OR = 1.43, 95%CI 1.26-1.62, P < 0.01) and more advanced TNM stage (OR = 1.80, 95%CI 1.56-2.07, P < 0.01).

Conclusion. Low LKB1 expression predicts shorter OS, worse tumor differentiation, larger tumors, earlier lymph node metastasis and more advanced TNM stage. Low LKB1 expression may be a useful biomarker of poor clinicopathology and prognosis.

Patient and public involvement statement. This systematic review does not need ethical approval. Results will be disseminated through conference presentations and publication in a peer-reviewed, scientific journal.

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 of tenmutated in lung, breast, gastric and other cancers [2-4]. LKB1 plays 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 tumor suppressor to restrict tumorigenesis and metastasis [8]. Mice with a 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 complex1 (mTORC1) and Hif-1, but through signalling involving pd-1 and TNF receptor proteins [9]. LKB1 deficiency can render tumor cells sensitive to metabolic stress, which may turn out to be an anti-tumor strategy [10].

Although several studies have examined the role of LKB1 in tumor inhibition, its role in the prognosis of solid tumors 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 tumors 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 favorable survival.

Therefore we systematically reviewed and meta-analyzed the relevant literature to understand

the current evidence about a relationship between LKB1 expression and prognosis in patients with solid tumors.

Materials and Methods

Literature search strategy

The following databases were searched from database establishment to June 15, 2018 to identify studies of LKB1 expression and survival in solid tumors: PubMed, Embase, Web of Science, Cochrance 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) LKB1expression in cancer tissue (obtained via surgery or biopsy) was measured by immunohistochemistry or Western blotting; (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 tumors; (3)sufficient data were published for calculating an odds ratio (OR) or hazard ratio (HR) and 95% confidence interval (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 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 analyze survival data; (3) tumor differentiation, tumor size, lymph node

metastasis and tumor 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 tumors 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

table. Correlation between LKB1 expression and clinicopathological characteristics of patients with solid tumors was evaluated in terms of OR and 95%CI.

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

I^2 was used to assess statistical heterogeneity. If $I^2 > 50\%$, heterogeneity was considered to exist among all included studies, and we conducted a subgroup analysis to investigate its possible source. If $I^2 < 50\%$, 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

testing 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 and Egger's test of survival data.

Results

A total of 4,858 potentially relevant studies were identified in literature searches, of which 3,374 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] (Fig 1). Data from all 25 studies were meta-analyzed to examine the potential correlation of LKB1 expression with clinicopathological characteristics. Data from 24 studies were meta-analyzed to examine the potential correlation between LKB1 expression and OS. Data from five studies were used to analyze 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 6,012 patients from six countries: China, USA, France, UK, Canada, and Egypt. Data on OS were reported in 24 studies, data on RFS in five studies, and data on DFS in four studies. Patients covered a range of cancers, including cancers of the lung, breast, prostate or pancreas; gastric cancer; hepatocellular carcinoma; esophagus squamous cancer; colorectal cancer; glioma; and laryngeal squamous cell carcinoma. Tables 1-2 summarize the characteristics of the included studies. Supplement table 1 lists clinicopathological characteristics and LKB1 expression. Eight studies had an NOS score of 8; 11studies, 7; 6 studies, 6; and 3 studies, 5 (supplement table 2 and

supplement 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-analyze the data. The pooled HR describing OS for patients with low LKB1 expression relative to OS for patients with high expression is shown in Fig 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 1.35-1.97, $P < 0.01$).

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 1.26-2.06, $P < 0.001$ with significant heterogeneity). This relationship was observed for the following cancer types: lung cancer (HR = 2.07, 95%CI 1.60-2.69, $P < 0.01$, I^2 =0%), pancreatic cancer (HR = 2.16, 95%CI 1.53-3.05, $P < 0.001$, I^2 = 0%), gastric cancer (HR = 2.11, 95%CI 1.60-3.01, $P < 0.01$, I^2=0%), and breast cancer (HR = 1.26, 95%CI 1.15-1.37,$P < 0.01$). However, this relationship was not observed in the case

of hepatocellular carcinoma (HR = 1.27, 95%CI 0.84-1.94, P = 0.26 with significant heterogeneity).

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

Pooled HR for the subgroup of patients tested by anti-LKB1 immunohistochemistry was 1.58 (95%CI 1.33–1.88, P < 0.01). Low LKB1 expression based on cytoplasmic staining predicted significant adverse prognosis (HR = 1.78, 95%CI 1.49-2.13, P < 0.01). This relationship was not observed when the judgment of low LKB1 expression was based on nuclear staining (HR = 1.25, 95%CI 0.85-1.85, 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 (Fig 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-analyzed using a random-effect model. Low LKB1 expression did not show a significant association with RFS based on univariate analysis (HR = 1.44, 95%CI 0.65-3.17, P = 0.37) or multivariate analysis (HR =

1.02, 95%CI 0.42-2.47, $P = 0.97$). Similarly, no significant correlation was observed between LKB1 expression and DFS based on univariate analysis and random-effect meta-analysis (HR = 1.49, 95%CI 0.73-3.01, $P = 0.27$) (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 0.57-1.05, $P = 0.10$) or sex (OR = 0.97, 95%CI 0.78-1.19, $P = 0.76$). In contrast, low LKB1 expression was significantly related to worse differentiation (OR = 1.17, 95%CI 1.14-2.55, $P < 0.01$), deeper invasion (OR = 1.68, 95%CI 1.24-2.27, $P < 0.01$), earlier lymph node metastasis (OR = 1.43, 95%CI 1.26-1.62, $P < 0.01$), and more advanced clinical stage (OR = 1.80, 95%CI 1.56-2.07, $P < 0.01$).

Results are shown as individual and pooled OR with 95% confidence intervals

Publication bias

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

Discussion

This meta-analysis suggests that among patients with many kinds of solid tumors, 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 unfavorable prognosis. In fact, the available evidence suggests an association of low LKB1 expression with worse tumor 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 co-morbidities 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 tumors, LKB1 acts as a tumor 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 tumors may be associated with up-regulation of glutamatede hydrogenase 1, which activates CamKK2 and its downstream effector AMPK to increase metastatic potential [38]. LKB1 loss may drive ovarian serous tumorigenesis 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 forLKB1 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 tumorigenic or tumor-suppressing molecule.

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 minimize the effects of such heterogeneity by using a random-effect 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 toward publication of

positive findings. Fifth, our meta-analysis did not account for numerous other factors that may also affect prognosis, such as co-morbidities 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 randomized controlled trials.

Conclusions

The available evidence links low LKB1 expression with poor prognosis in patients with various types of solid tumors. 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.

Contributors: XMY and HYM designed the study. FJZ, HYM and RRJ conduct systematic search, search literature and extract data. YHR analyzed 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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Data sharing statement: No additional data are available.

Legends:

Fig 1. Flow diagram of the eligible studies

Fig 2. Forest plot of the association between decrease LKB1 expression and OS

Fig 3. Sensitivity analysis of OS in the meta-analysis

Fig 4. Funnel plot for the potential publication bias

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Table1. Main characteristics of included studies and Newcastle-Ottawa scale scores.

Study	Year	Country	Type of cancer	No. cases		Age in year, median(range)	Follow-up, mo.	NOS score
				Low LKB1	High LKB1			
Ding XM	2005	China	Lung adenocarcinoma	24	38	60.5(32–77)	80	7
Tsai LH	2013	China	Lung adenocarcinomas	44	71	NR	140	7
Jiang LL	2014	China	Non-small cell lung cancer	33	109	58.2(31-84)	71	7
Calles A	2015	USA	Lung adenocarcinoma	42	84	63.5(30-84)	60	7
Shen Z	2002	China	Breast carcinoma	38	83	53.7(32-77)	70	6
Bouchekioua-Bouzaghou K	2014	France	Breast cancer	94	60	56.87(27-87)	162	7
Bouchekioua-Bouzaghou K	2014	France	Breast cancer	102	52	56.5 (27-87）	162	
Chen IC	2016	China	Breast cancer	161	408	48	120	6
Chen IC	2016	China	Breast cancer	88	189	54	120	
Chen IC	2016	UK and Canada	Breast cancer	494	494	61.3	300	5
Chen IC	2016	UK and Canada	Breast cancer	488	487	62.6	300	
HamdyA.Azim	2016	Egypt	Breast Cancer	12	20	51.3(25-82)	82.8	6
HamdyA.Azim	2016	Egypt	Breast Cancer	11	21	51.3(25-82)	82.8	
Morton JP	2010	UK	Pancreatic cancer	20	86	NR	95	7
Yang JY	2015	China	Pancreatic ductal adenocarcinoma	36	169	NR	97	8

Li DZ	2018	China	Pancreatic neuroendocrine tumor	38	33	NR	190	8
Yang XW	2012	China	Gastric Cancer	76	24	65(31-85)	38	7
Huang Y	2014	China	Gastric carcinoma	24	91	61(37-80)	75	6
Ma LG	2016	China	Gastric Cancer	62	47	57(31-84)	99	8
Sun JJ	2016	China	Gastric Cancer	107	48	NR	70	6
Yin M	2017	China	Gastric Cancer	78	32	62(23-79)	72	7
Huang YH	2013	China	Hepatocellular carcinoma	31	39	57(43-72)	68	7
Lee SW	2015	China	Hepatocellular carcinoma	13	27	NR	101	7
Wu CC	2018	China	Hepatocellular carcinoma	41	52	NR	54	7
Wang JH	2015	China	Intrahepatic cholangiocarcinoma	187	129	NR	99	8
Ma JJ	2014	China	Esophagus squamous cancer	73	47	NR	60	8
He TY	2014	China	Colorectal cancer	63	95	NR	80.5	5
Lu JL	2015	China	Prostate Cancer	78	31	NR	60	7
Huang JH	2017	China	Glioma	92	88	50.8(10-86)	118	8
He SS	2017	China	Laryngeal squamous cell carcinoma	128	80	NR	212.2	8

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	IHC	Both nucleus and cytoplasm	Lower than in normal airway epithelium	OS	UA	3.003(1.524-5.865)
Tsai LH	IHC	No specific description	A score equal to or lower than 100	OS	UA	1.846(1.147-2.952)
					MA	1.868(1.160-3.007)
				RFS	UA	1.828(1.247-3.122)
					MA	1.791(1.132-2.834)
Jiang LL	IHC	Cytoplasm	Score of 0-4	OS	UA	3.226(1.856-5.586)
					MA	2.128(1.136–4.000)
Calles A	IHC	Cytoplasm	No staining	OS	UA	1.440(0.910–2.270)
ShenZ	WB	Total protein	Bands of the breast cancer tissue in which the quantities were <0.5	OS	UA	3.754(1.583-8.932)
				DFS	UA	2.529(1.383-5.933)
Bouchekioua-Bouzaghou K	IHC	Cytoplasm	Staining intensity recorded as 0-1	OS	UA	0.418(0.211-0.828)
					MA	0.403(0.199-0.820)
				DFS	UA	0.495(0.249-0.809)
					MA	0.549(0.303-0.990)
Bouchekioua-Bouzaghou K	IHC	nucleus	Staining intensity recorded as 0	OS	UA	1.417(0.722-2.734)
				DFS	UA	1.278(0.732-2.225)
Chen IC	IHC	No specific description	A score of 0 or 1	OS	UA	1.200(0.670-2.150)
					MA	0.766(0.453–1.296)
Chen IC	IHC	No specific description	A score of 0 or 1	OS	UA	0.980(0.600-1.610)
					MA	1.054(0.665-1.671)
Chen IC	microarray data	No specific description	Lower than the median expression level	OS	UA	1.600(1.360-1.894)
					MA	0.937(0.772–1.138)
Chen IC	microarray data	No specific description	Lower than the median expression level	OS	UA	1.090(0.910-1.300)
					MA	1.024(0.839–1.250)

HamdyA.Azim	IHC	Cytoplasm	Staining intensity recorded as 0	RFS	UA	1.110(0.160-7.490)
					MA	0.810(0.220-3.030)
HamdyA.Azim	IHC	Nucleus	Staining intensity recorded as 0	RFS	UA	5.220(0.23-118.460)
					MA	0.360(0.150-0.100)
Morton JP	IHC	Cytoplasm	Histoscore was≤100	OS	UA	1.877(1.020–3.448)
					MA	1.870(1.090–3.220)
Yang JY	IHC	No specific description	A total score<4	OS	UA	2.278(1.495–3.472)
					MA	1.845(1.189–2.856)
LiZDZ	IHC	Cytoplasm	Strong immunostaining in ≤50% of the cells and/or weak staining	OS	UA	5.310(0.200-142.482)
				DFS	UA	2.190(0.410-11.700)
Yang XW	IHC	Both nucleus and cytoplasm	Staining intensity in the neoplasmless than that innormal mucosa	OS	UA	2.558(1.554-4.233)
Huang Y	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	2.514(1.260–5.022)
Xu LG	IHC	Both nucleus and cytoplasm	Scores ≤1	OS	UA	2.310(1.250-4.270)
					MA	3.527(1.491-10.630)
Sun JJ	IHC	Both nucleus and cytoplasm	Scores of 0 and 1+ indicate negative result	OS	UA	1.450(0.540-3.900)
					MA	4.431(1.363-14.407)
Yin M	IHC	Both nucleus and cytoplasm	Staining intensity recorded as 0–1	OS	UA	1.070(0.460-2.470）
Huang YH	IHC	Cytoplasm	Staining index score≤ 3	OS	UA	3.155(1.603-6.211)
					MA	2.179(1.066-4.44)
				DFS	UA	2.737(1.629-6.271)
Lee SW	IHC	Both nucleus and cytoplasm	H-score was lower than the median	OS	UA	0.517(0.284–0.931)
					MA	0.333(0.193–0.564)
Wu CC	IHC	No specific description	Histoscore was≤150	OS	UA	3.130(0.910-10.840)
					MA	4.260(1.870-9.690)
				RFS	UA	2.020(0.870-4.720)
					MA	2.050(1.110-3.810)

Wang JH	IHC	Cytoplasm	Staining density lower than the median value	OS	UA	1.857(1.438–2.386)
					MA	1.824(1.404–2.377)
Ma JJ	IHC	Both nucleus and cytoplasm	Score of 0–4	OS	UA	0.570(0.330-0.980)
Hu TY	IHC	No specific description	Score equal to or lower than 100	OS	UA	2.364(1.466-3.812)
					MA	3.146(1.876-5.276)
				RFS	UA	2.522(1.701-4.445)
					MA	3.093(1.843-5.191)
Lu JL	IHC	No specific description	Staining of fewer than 20% of the tissue cells or no staining	OS	UA	6.310(0.420-94.730)
					MA	3.981(1.698–9.336)
Huang JH	IHC	No specific description	Percentage of positive cells ⩽ 35%and/or staining intensity score of 0-1.	OS	UA	3.350(1.490-7.510)
					MA	3.022(1.002-6.016)
He SS	IHC	Nucleus	Score ⩽4	OS	UA	1.170(0.720-1.900)
					MA	1.628(1.060–2.500)

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.

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

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

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

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)	0.78(0.57-1.05)	$P = 0.10$	4.04	0%	0.78
Sex(Male, Female)	0.97(0.78-1.19)	$P = 0.76$	9.06	0%	0.77
Tumor differentiation(Poor, Well)	1.17(1.14-2.55)	$P < 0.01$	59.5	75%	<0.001
Tumor size(T3-T4,T1-T2)	1.68(1.24-2.27)	$P < 0.01$	43.34	61%	<0.001
Lymph node metastasis(Yes, No)	1.43(1.26-1.62)	$P < 0.01$	58.41	74%	<0.001
TNM stage(Ⅲ-Ⅳ, Ⅰ-Ⅱ)	1.80(1.56-2.07)	$P < 0.01$	88.8	81%	<0.001

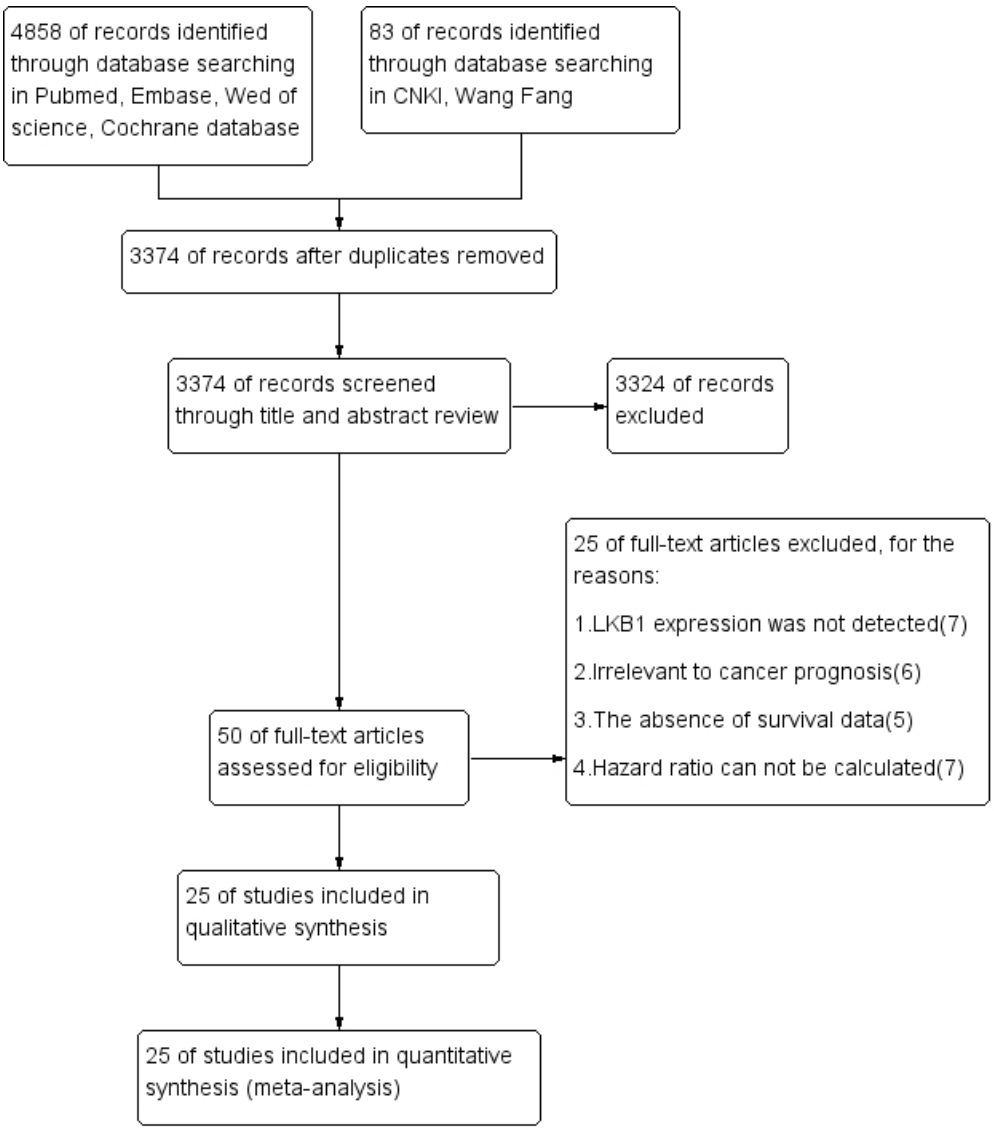

Fig 1. Flow diagram of the eligible studies

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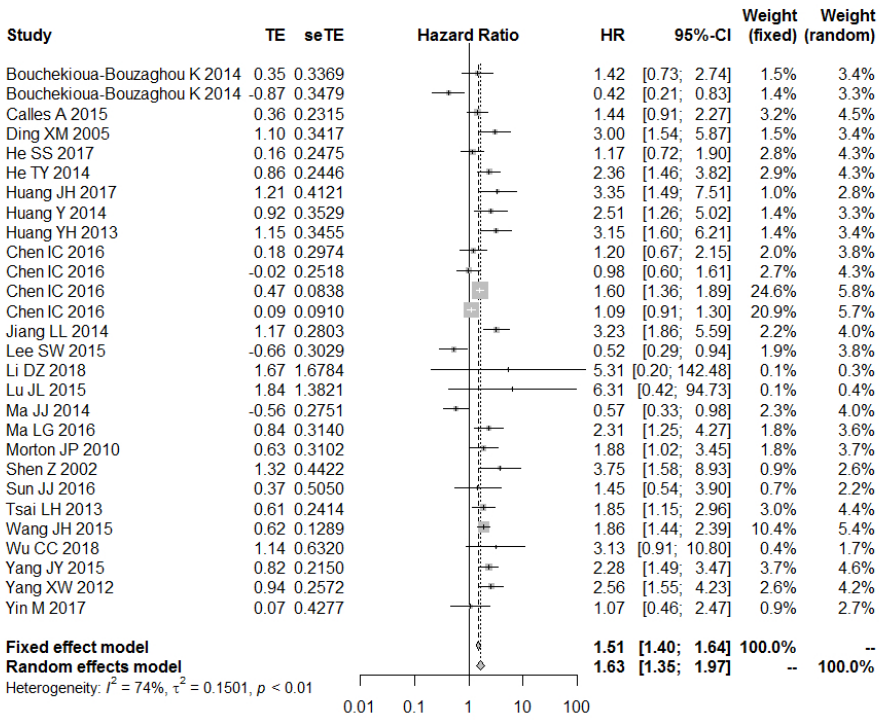

Fig 2. Forest plot of the association between decrease LKB1 expression and OS.

Fig 2. Forest plot of the association between decrease LKB1 expression and OS

257x211mm (96 x 96 DPI)

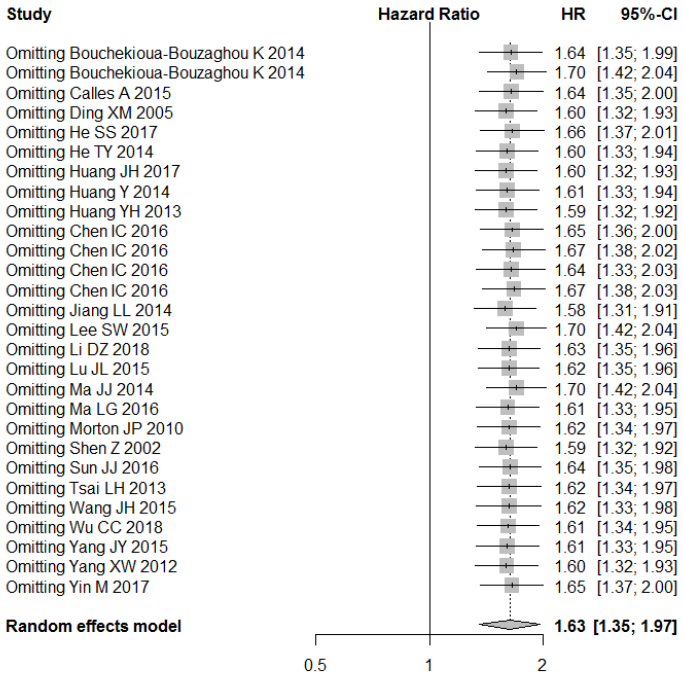

Fig 3. Sensitivity analysis of OS in the meta-analysis

Fig 3. Sensitivity analysis of OS in the meta-analysis

257x211mm (96 x 96 DPI)

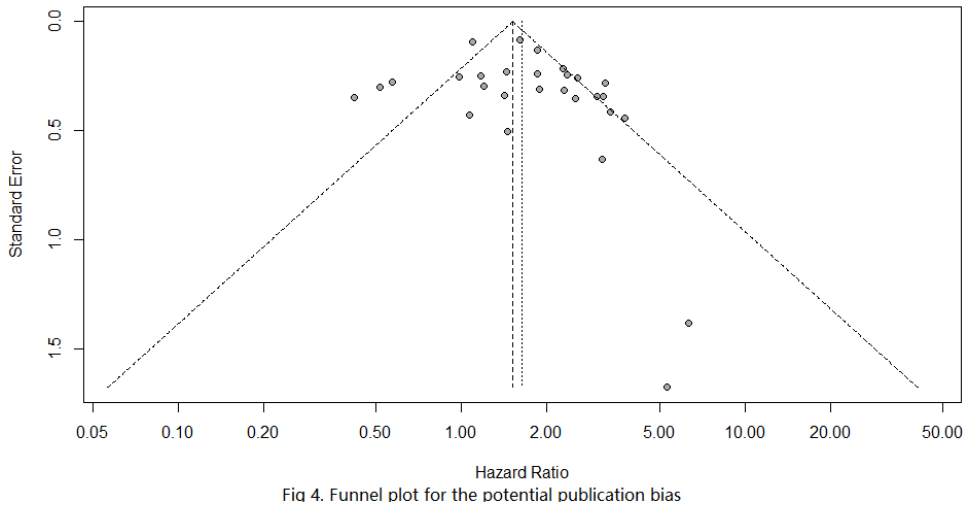

Fig 4. Funnel plot for the potential publication bias

257x148mm (96 x 96 DPI)

Supplement table 1. LKB1 expression and clinicopathological characteristics.

Study	LKB1 expression	Age		Sex		Tumor differentiation		Tumor size		Lymph node metastasis		TNM stage	
		⩾60	<60	Male	Female	Poor	Well	T3-T4	T1-T2	Yes	No	Ⅲ-Ⅳ	Ⅰ-Ⅱ
Huang YH	Low	-	-	26	5	23	8	15	16	-	-	19	12
	High	-	-	31	8	17	22	20	19	-	-	27	12
He TY	Low	-	-	-	-	-	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-	-	-	-	-	-
Bouchekioua-Bouzaghou K	Low (cytoplasmic staining)	-	-	-	-	69	25	26	68	50	44	-	-
	High	-	-	-	-	54	6	18	42	38	22	-	-
	Low(nuclear staining)	-	-	-	-	83	19	34	68	63	39	-	-
	High	-	-	-	-	40	12	10	42	25	27	-	-
ShenZ	Low	-	-	-	-	35	3	13	25	-	-	-	-
	High	-	-	-	-	69	14	11	73	-	-	-	-
Tsai LH	Low	-	-	25	19	-	-	9	35	35	9	28	16
	High	-	-	41	30	-	-	9	62	31	40	24	47
Jiang LL	Low	16	17	17	16	23	10	-	-	18	15	12	21
	High	49	60	65	44	34	75	-	-	44	65	23	86
Yang JY	Low	-	-	16	20	32	4	35	1	17	19	16	20
	High	-	-	101	68	159	10	132	37	45	124	31	138
Calles A	Low	-	-	14	28	-	-	-	-	-	-	-	-
	High	-	-	25	59	-	-	-	-	-	-	-	-
Wang JH	Low	-	-	122	65	162	25	112	100	76	111	117	70
	High	-	-	93	46	90	49	63	51	42	97	56	83
Morton JP	Low	-	-	-	-	-	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-	-	-	-	-	-
Ding XM	Low	12	12	13	11	-	-	9	15	3	21	22	2
	High	21	17	14	24	-	-	7	31	8	20	15	23
Yang XW	Low	52	24	60	16	62	14	57	19	59	17	48	19
	High	16	8	20	4	20	4	13	11	7	17	6	11
Wu CC	Low	17	24	32	9	-	-	-	-	-	-	26	15
	High	25	27	45	7	-	-	-	-	-	-	32	20
Yin M	Low	43	35	54	24	57	21	71	7	56	22	68	20
	High	19	13	23	9	12	20	24	8	11	21	12	20

Huang Y	Low	51	17	-	-	-	-	65	26	64	27	80	11
	High	40	7	-	-	-	-	16	8	8	16	10	14
Ma LG	Low	51	22	60	13	60	13	48	25	24	49	31	42
	high	36	11	36	11	24	23	24	23	5	42	6	41
Chen IC	low	-	-	-	-	126	25	16	145	67	91	23	138
	High	-	-	-	-	311	81	34	372	152	253	57	351
	Low	-	-	-	-	83	2	6	82	56	32	36	52
	High	-	-	-	-	177	12	15	174	106	83	66	123
	Low	-	-	-	-	457	37	20	474	241	253	85	369
	High	-	-	-	-	459	35	26	468	235	259	88	406
	Low	-	-	-	-	392	49	29	451	241	238	88	393
	High	-	-	-	-	398	46	24	446	213	260	85	388
Li DZ	Low	-	-	26	12	6	32	-	-	-	-	-	-
	High	-	-	16	17	1	32	-	-	-	-	-	-
Huang YH	Low	-	-	56	36	-	-	52	40	-	-	77	15
	High	-	-	54	34	-	-	20	68	-	-	43	55
HamdyA. Azim	Low	-	-	-	-	-	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-	-	-	-	-	-
Lu JL	Low	-	-	-	-	-	-	-	-	-	-	47	31
	High	-	-	-	-	-	-	-	-	-	-	11	20
He SS	Low	-	-	-	-	-	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-	-	-	-	-	-
Ma JJ	Low	29	33	40	22	27	35	44	18	51	11	28	34
	High	30	17	31	16	19	28	18	29	29	18	9	38
Lee SW	Low	-	-	-	-	-	-	-	-	-	-	-	-
	High	-	-	-	-	-	-	-	-	-	-	-	-
Sun JJ	Low	60	47	79	28	78	29	73	34	60	47	55	52
	High	28	20	42	6	38	10	22	26	16	32	8	40

S2 Table. Newcastle-Ottawa Scale (NOS) for quality assessment in meta-analysis.

Selection
(1) Representativeness of the exposed cohort
(a) Truly representative of the cancer patients in the community (1 star)
(b) Somewhat representative of the cancer patients in the community (1 star)
(c) Selected group of users (e.g., nurses, volunteers)
(d) No description of the derivation of the cohort
(2) Selection of the non-exposed cohort
(a) Drawn from the same community as the exposed cohort (1 star)
(b) Drawn from a different source
(c) No description of the derivation of the non-exposed cohort
(3) Ascertainment of exposure (proof of cancer and LKB1 measurement)
(a) Secure record (e.g., surgical records or pathological diagnosis) (1 star)
(b) Structured interview (1 star)
(c) Written self-report
(d) No description
(4) Demonstration that outcome of interest was not present at start of study
(a) Yes (1 star)
(b) No
Comparability
(1) Comparability of cohorts based on the design or analysis
(a) The age between exposed cohort and non-exposed cohort had no significant difference (1 star)
(b) The sex (or grade, stage, etc.) between exposed cohort and non-exposed cohort had no significant difference (1 star)
Outcome
(1) Assessment of outcome (death or recurrence)
(a) Independent blind assessment (1 star)
(b) Record linkage (1 star)
(c) Self-report
(d) No description
(2) Was follow-up long enough for outcomes to occur? (death or recurrence)
(a) Yes (at least 3 years) (1 star)
(b) No
(3) Adequacy of follow-up of cohorts
(a) Complete follow-up—all subjects accounted for (1 star)
(b) Subjects lost to follow-up unlikely to introduce bias—small number lost (less than 25%) or description provided of those lost (1 star)
(c) Follow-up rate less than 75% and no description of those lost
(d) No statement

Note: a maximum of one "star" for each item within the "Selection" and "Outcome" categories, maximum of two "stars" for "Comparability".

S3 Table. Quality assessment of the 25 included studies according to the NOS.

Study	Year	Country	Type of cancer	Selection/4	Comparability/2	Outcome/3	Total score
Ding XM	2005	China	Lung adenocarcinoma	1+1+1+0=3	1+1=2	1+1+1=3	8
Tsai LH	2013	China	Lung adenocarcinomas	1+1+1+0=3	1+1=2	1+1+0=2	7
Jiang LL	2014	China	Non-small cell lung cancer	1+1+1+0=3	1+1=2	1+1+0=2	7
Calles A	2015	USA	Lung adenocarcinoma	1+1+1+0=3	1+1=2	1+1+0=2	7
Shen Z	2002	China	Breast carcinoma	1+1+1+0=3	1+0=1	1+1+0=2	6
Bouchekioua-Bouzaghou K	2014	France	Breast cancer	1+1+1+0=3	1+1=2	1+1+0=2	7
Chen IC	2016	China	Breast cancer	1+1+1+0=3	1+0=1	1+1+0=2	6
		UK and Canada	Breast cancer	1+1+1+0=3	0+0=0	1+1+0=2	5
HamdyA.Azim	2016	Egypt	Breast Cancer	1+1+1+0=3	0+0=0	1+1+0=2	6
Morton JP	2010	UK	Pancreatic cancer	1+1+1+0=3	0+0=0	1+1+1=3	6
Yang JY	2015	China	Pancreatic ductal adenocarcinoma	1+1+1+0=3	1+1=2	1+1+0=2	7
Li DZ	2018	China	Pancreatic neuroendocrine tumor	1+1+1+0=3	1+1=2	1+1+1=3	8
Yang XW	2012	China	Gastric Cancer	1+1+1+0=3	1+1=2	1+1+0=2	7
Huang Y	2014	China	Gastric carcinoma	1+1+1+0=3	1+0=1	1+1+0=2	6
Ma LG	2016	China	Gastric Cancer	1+1+1+0=3	1+1=2	1+1+1=3	8
Sun JJ	2016	China	Gastric Cancer	1+1+1+0=3	1+0=1	1+1+0=2	6
Yin M	2017	China	Gastric Cancer	1+1+1+0=3	1+0=1	1+1+1=3	7
Huang YH	2013	China	Hepatocellular carcinoma	1+1+1+0=3	1+1=2	1+1+0=2	7
Lee SW	2015	China	Hepatocellular carcinoma	1+1+1+0=3	1+1=2	1+1+0=2	7
Wu CC	2018	China	Hepatocellular carcinoma	1+1+1+0=3	1+0=1	1+1+1=3	7

Wang JH	2015	China	Intrahepatic cholangiocarcinoma	1+1+1+0=3	1+1=2	1+1+1=3	8
Ma JJ	2014	China	Esophagus squamous cancer	1+1+1+0=3	1+1=2	1+1+1=3	8
He TY	2014	China	Colorectal cancer	1+1+1+0=3	0+0=0	1+1+0=2	5
Lu JL	2015	China	Prostate Cancer	1+1+1+0=3	1+0=1	1+1+1=3	7
Huang JH	2017	China	Glioma	1+1+1+0=3	1+1=2	1+1+1=3	8
He SS	2017	China	Laryngeal squamous cell carcinoma	1+1+1+0=3	1+1=2	1+1+1=3	8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a meta-analysis.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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