PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between LKB1 expression and prognosis of patients with solid tumors: an updated systematic review and meta-analysis
AUTHORS	Ren, Yun; Zhao, Feng; Mo, Han; Jia, Rong; Tang, Juan; Zhao, Xin; Wei, Jue; Huo, Rong; Li, Qiu; You, Xue

VERSION 1 - REVIEW

REVIEWER	jay Whelan University of Tennessee, Knoxville, TN, USA
REVIEW RETURNED	21-Nov-2018

GENERAL COMMENTS	This paper by Ren et al. investigates the impact of LKB1 expression in a variety cancers on survival (overall, disease-free, recurrence-free) and a number of other clinical parameters. This is a very important area of research as LKB1 has been proposed to be a tumor suppressor protein where its mutation is associated with poor prognosis. This paper performs a meta-analysis of relevant studies that explore expression patterns of LKB1 in a variety of human cancers on relevant outcomes. Their work supports the existing literature that proposes a beneficial effect when LKB1 expression is higher. This is critical because despite the same downstream target as CaMKK2, that being AMPK, there appears to be an antithetic relationship between these two kinases with regards to cancer prognosis. This paper solidifies this relationship in many cancers (particularly because CaMKK is typically over expressed). It is not clear if the authors searched the Cochrane Clinical Trial Database or other clinical trial registries for ongoing trials that have published preliminary results. This could pick up smaller trials that are missing from their data set. They should also clarify the dates for their search (it just says through June 15, 2018).

REVIEWER	Yi PS North Sichuan Medical College, China
REVIEW RETURNED	24-Jan-2019

GENERAL COMMENTS	The study by Ren et al was designed to investigate the relation between LKB1 expreesion and survival of solid tumors. However, Xiao et al published a similar study in 2016, compared with the

	previous study, the current study have not got any interesting findings, moreover, the language is poor written, there are variety of language errors in main contents. Regarding to the methods, the authors included 25 studies for meta-analysis, the patients varied in coutries, pathological types, and general status, the pooled results is not meaningful for clincal practice, in addition, as the author presented, they detected obvious heterogeneity among included studies. In the analysis of relation between LKB1 and clinicopathological features of tumors, the authors simplely classified tumors according to TNM staging system. However, not all solid tumors are qualified for TNM staging, for instance, the novel staging system is BCLC staging, which is widely accepted by researchers, if the pooled results just based on TNM staging, patients with HCC should not be included. In a word, this study is poorly designed and have not obtained any important or interesting results, we suggest rejecting for publication.

REVIEWER	Cristian Ricci North-West University, South Africa
REVIEW RETURNED	22-Feb-2019

GENERAL COMMENTS	The present manuscript report about the association between LKB1 expression and diagnosis of solid tumors assessed using a systematic review and meta-analysis. The topic could be considered of general medical interest but some major points should be addressed. As a statistical reviewer I will only focus on main methodological aspects of the statistical analysis. 1) A relevant heterogeneity was observed for all of the meta-analysis reported. This variability should be addressed by means of meta-regression to define/identify the possible determinants of heterogeneity. Notably, not only "biological" but also methodological (study design, NOS score...) possible source of variation should be taken into account. Table 4 should be extended. 2) I think that a non-linear-dose response analysis could be of interest. To this aim, the work from Greenland and Longnecker should be taken into account. Greenland, S., Longnecker, M. P. (1992). Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. American journal of epidemiology, 135(11), 1301-1309. A suitable method was described by Orsini and colleagues Orsini, N., Li, R., Wolk, A., Khudyakov, P., Spiegelman, D. (2012). Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. American journal of epidemiology, 175(1), 66-73. 3) A much more formal test for the assessment of publication bias should be performed. To this purpose I would consider to perform at least both Egger's and Begg's tests. Nevertheless the funnel plot looks skewed and a publication bias could be confirmed by those tests 4) Adjusting factors considered by the single studies to perform RR estimates should be reported. A second step would be to perform a dedicated sensitivity analysis and metaregression considering the level of adjustment (for example basic (gender and

	age and other simple factors) vs more comprehensive (considering also alcohol, or other behavioural factors…) Minor: there are a number of uncommon elements (HR reported with 3 decimals) and some sentences that may need a professional editing by a mother tongue English scientist

REVIEWER	Sadik Khuder Department of Medicine University of Toledo 3120 Glendale Ave Toledo, OH, United States
REVIEW RETURNED	23-Feb-2019

GENERAL COMMENTS	1. HR and OR need to be separated from the numbers. I suggest including "=" between HR or OR and the numbers. For example: OR = 0.78, 95%CI etc. 2. Abstract: Strength and limitations of this study. Rewrite this part to coincide with the text in the discussion.

VERSION 1 – AUTHOR RESPONSE

Comments to the Author

Reviewer: 1

1.It is not clear if the authors searched the Cochrane Clinical Trial Database or other clinical trial registries for ongoing trials that have published preliminary results. This could pick up smaller trials that are missing from their data set. They should also clarify the dates for their search (it just says through June 15, 2018).

Response: We searched 20 clinical studies from the Cochrane clinical trial database, which were carefully screened and excluded.

2.They should also clarify the dates for their search (it just says through June 15, 2018).

Response: We identified the date for our search(From database establishment to June 15, 2018).

Reviewer: 2

1. Xiao et al published a similar study in 2016, compared with the previous study, the current study have not got any interesting findings, moreover, the language is poor written, there are variety of language errors in main contents.

Response: Xiao's meta-analysis enrolled 14 studies, including 7 cancer types. However, our systematic review included 25 studies containing 6,012 patients and 9 cancer types. Our study has a larger sample sizes and more detailed subgroup analysis than Xiao's study. Our work supports the existing literature that proposes a beneficial effect when LKB1 expression is higher.

2. Regarding to the methods, the authors included 25 studies for meta-analysis, the patients varied in coutries, pathological types, and general status, the pooled results is not meaningful for clincal

practice, in addition, as the author presented, they detected obvious heterogeneity among included studies.

Response: According to Region, Cancer type, Staining position and NOS scores, we conducted a subgroup analysis to define/identify the possible determinants of heterogeneity. This association of LKB1 expression and prognosis was observed for the following cancer types: lung cancer, pancreatic cancer, gastric cancer with the disappearance of heterogeneity. However, this association was not observed in the case of hepatocellular carcinoma with significant heterogeneity.

3. In the analysis of relation between LKB1 and clinicopathological features of tumors, the authors simplely classified tumors according to TNM staging system. However, not all solid tumors are qualified for TNM staging, for instance, the novel staging system is BCLC staging, which is widely accepted by researchers, if the pooled results just based on TNM staging, patients with HCC should not be included. In a word, this study is poorly designed and have not obtained any important or interesting results, we suggest rejecting for publication.

Response: Thank you for pointing this out. We reviewed our included literatures and consulted relevant clinical experts. We cautiously decided to adopt TNM staging, because most of the studies we included used TNM stadge, but not BCLC stadge.

Reviewer: 3

1.A relevant heterogeneity was observed for all of the meta-analysis reported. This variability should be addressed by means of meta-regression to define/identify the possible determinants of heterogeneity.

Response: We analyzed the heterogeneity of the study by subgroup analysis to define/identify the possible determinants of heterogeneity. According to cancer type, the heterogeneity of pool HRs for lung cancer, gastric cancer and pancreatic cancer were disappeared, but the hepatocellular carcinoma was significant. In the staining position subgroup, the pool HRs for nucleus have less heterogeneity but the group of nucleus and cytoplasm was significant. In addition, we reconsidered the possible influence of NOS score on heterogeneity and performed the subgroup analysis.

2. I think that a non-linear-dose response analysis could be of interest. To this aim, the work from Greenland and Longnecker should be taken into account.

Response: We have attempted non-linear-dose-response analysis after reading the references you gave. However, the cut-off value of LKB1 among the included studies were inconsistent. It seems that our data cannot be used for non-linear-dose-response analysis after consulting relevant statistical experts.

3. A much more formal test for the assessment of publication bias should be performed.

Response: We supplemented the Begg’s and Egger’s test to assess the publication bias.

4. Adjusting factors considered by the single studies to perform RR estimates should be reported. A second step would be to perform a dedicated sensitivity analysis and meta-regression considering the level of adjustment (for example basic (gender and age and other simple factors) vs more comprehensive (considering also alcohol, or other behavioural factors…)

Response: In order to ensure the accuracy of our data, all HRs and 95%CI were extracted and cross-checked by two researchers, and disagreements were resolved by a third reviewer. In addition,we added sensitivity analysis showing in figure 3.

Reviewer: 4

1. HR and OR need to be separated from the numbers. I suggest including "=" between HR or OR and the numbers. For example: OR = 0.78, 95%CI etc.

Response: We added the " = " between HR or OR and the numbers.

2. Abstract: Strength and limitations of this study. Rewrite this part to coincide with the text in the discussion.

Response: We have re-written the Strength and limitations of this study according to the Reviewer's suggestion.

VERSION 2 – REVIEW

REVIEWER	Jay Whelan The University of Tennessee at Knoxville Knoxville, TN, USA
REVIEW RETURNED	17-Apr-2019

GENERAL COMMENTS	1. The significant figures for the HR in figure 2 needs to be standardized. Do you need all those significant figures? 2. There are a lot of grammatical and typographical errors. So much so, I can't even list the number of these errors because there are so many.

REVIEWER	Cristian Ricci North-West University
REVIEW RETURNED	12-Apr-2019

GENERAL COMMENTS	The authors did their best to address the most of my comments, most of which cannot be addressed because of the nature of the included studies. The work is barely sufficient from a statistical viewpoint but may be of scientific interest. I recommend to accept if comments from other reviewers are fully addressed.