Prognostic Significance of Pyruvate Kinase M2 Expression in Esophageal Squamous Cell Carcinoma and Its Meta-Analysis

Qiqi Zhang

The Institute of Clinical Research <https://orcid.org/0000-0002-0734-7328>

Shutao Zheng

Institute for Medical Research

Qing Liu

Institute for Medical Research

Tao Liu

Institute for Medical Research

Aerziguli Tuerxun

Institute for Medical Research

Lifei Yang

Institute for Medical Research

Xiujuan Han

Institute for Medical Research

Xiaomei Lu (✉ luxiaomei88@163.com)

Institute for Medical Research

Research

Keywords: Esophageal squamous cell carcinoma (ESCC), Pyruvate kinase 2 (PKM2), Overall survival (OS), Meta-analysis, Prognosis.

Posted Date: March 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-263600/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Pyruvate kinase 2 (PKM2) is a key enzyme in glycolysis pathway and has been reported to be associated with the development of esophageal squamous cell carcinoma (ESCC). However, the prognostic value of PKM2 in ESCC remains undetermined.

Materials and methods: This study aimed to investigate the clinicopathological significance of PKM2 expression in ESCC. A comprehensive and systematic literature search was conducted using PubMed, Embase, Medline and Cochrane library databases. Appraised were the quality of studies, the potential for bias, and performed meta-analysis to assess the prognostic impact of PKM2 on the overall survival (OS).

Results: Five studies with 781 patients were eligible and enrolled eventually. Patients with high PKM2 expression were associated with poor prognosis in ESCC (HR=1.72, 95%CI: 1.41~2.09, $P=0.01$). Furthermore, up-regulated PKM2 was significantly associated with lymph node metastasis (OR=2.38, 95%CI: 1.68~3.35, $P=0.01$), clinical stage (OR=3.29, 95%CI: 2.27~4.77, $P=0.01$) and T classification (OR=2.92, 95%CI: 2.05~4.16, $P=0.01$).

Conclusions: In conclusion, high PKM2 expression denotes worse OS in ESCC patients, and correlate with the lymph node metastasis, clinical stage and T classification. However, further studies are warranted to assess how PKM2 can be implemented as a reliable staging element in clinical practice and whether it could provide a new target for therapeutic intervention.

Background

Esophageal cancer (EC) is one of the most common gastrointestinal tract tumors. According to the most recent International Agency for Research on Cancer (IARC) report, 455,800 new EC cases (3% of all cancers) were reported and 400,200 deaths due to EC (5% of all cancer deaths) occurred worldwide in 2012[1]. China is one of the highest rates of EC in the world, with an average of about 150,000 deaths per year. Esophageal squamous cell carcinoma (ESCC) is the main type in China[2]. The clinical treatment methods include surgical treatment, radiotherapy, chemotherapy, endoscopic treatment, traditional Chinese medicine anti-tumor treatment, palliative treatment, etc. Although these traditional treatments have been used in clinical practice for many years, the prognosis of patients with ESCC is still poor. The overall 5-year survival rate was 18 percent[3]. Therefore, new efficient prognostic markers are needed to be identified for risk estimation in ESCC patients.

Numerous oncological studies focus on cancer metabolism due to the aberrant feature of energy production. Tumor cells acquire the vast majority of energy from glycolysis and lactic acid fermentation regardless of sufficient oxygen supply, this unique phenomenon is known as Warburg effect, or aerobic glycolysis[4]. Pyruvate kinase (PK) is one of the main rate-limiting enzymes in glycolysis. There are two isoenzymes, M-type and L-type, and M-type has M1 and M2 isoforms (PKM1 and PKM2). PKM1 and PKM2 are different in expression and function[5]. PKM2 is mainly expressed in differentiated tissues, such as lung, adipose tissue, retina and islet, and is also expressed in cells with high nucleic acid synthesis rate, such as normal proliferating cells, embryonic cells and tumor cells[6]. Several findings caught researcher's attention to the potential role of PKM2 in tumorigenesis. Sizemore et al[7] confirmed that increased nuclear pT328-PKM2 level is associated with significantly worse survival in glioblastoma patients, so the use of PKM2-targeting strategies as a means to not only disrupt cancer metabolism but also inhibit an important mechanism of resistance to genotoxic therapies. For ESCC, PKM2 is over-expressed in ESCC tissues and cell lines[8], and PKM2 over-expression is associated with poor prognosis of ESCC[9–14].

However, the clinical significance of PKM2 expression remains controversial because of conflicting clinical evidence. Hence, we performed this meta-analysis to clarify the prognostic significance of PKM2 in ESCC and offer referential information for future clinical practice.

Materials And Methods

Search strategy

This meta-analysis was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses[15]. The PubMed, Embase, Medline, and Cochrane Library were searched with the key phrases “esophageal squamous cell carcinoma OR oesophageal squamous cell carcinoma OR ESCC” AND “pyruvate kinase M2 OR PKM2”, and the Google academic and latest conferences were also searched.

Selection criteria

The required literature inclusion criteria: (1) All patients in the original study were histopathologically diagnosed with ESCC; (2) Assessment of the expression of PKM2 with immunohistochemistry (IHC); (3) Analysis of the associations of PKM2 with disease-specific survival (DSS), disease-free survival (DFS), progression-free survival (PFS), or overall survival (OS); (4) Full text available. Exclusion criteria: Kaplan-Meier survival curve was not used for survival analysis in the original literature, or the data were incomplete and hazard ratio (HR) data could not be obtained.

Literature screening, data extraction and quality evaluation

Two investigators (Qiqi Zhang and Shutao Zheng) independently screened the retrieved literature by title and abstract for inclusion in the review. If the suitability of an article was uncertain, the full text was assessed. Disagreements were resolved by consensus or reviewed by a third investigator (LIFEI Yang). The following information were extracted from the studies that were enrolled: general information (including the first author's name, published year, name of the literature, the details of publication), the number of patients, clinical features of patients, detection method, antibody, cut-off value of high and low expression of PKM2, high expression rate of PKM2, overall survival (OS), estimate the hazard ratio (HR), 95% confidence interval (CI). If HR is not directly provided in the original literature, a request is sent to the author via email. If the authors do not respond, the data is extracted from the survival

curve. The Newcastle-Ottawa Scale (NOS) was used by the two investigators to assess the quality of the original studies. Disagreements were resolved by consensus or reviewed by the third investigator. NOS evaluates randomized, case-control, and cohort studies by evaluating population selection, comparability, exposure evaluation, or outcome evaluation. It contains 8 entries, and the evaluation of literature quality is based on the semi-quantized principle of the star system with a full score of 9. All studies with NOS scores of 6 or above are considered to be of high quality.

Statistical analysis

Review Manager Software 5.3 (Cochrane Collaborative, Oxford, UK) was used in the meta-analysis. Results can be presented graphically with the software. Heterogeneity between studies was assessed by χ^2 -based Q test and I^2 tests, where $I^2(\%) > 50\%$ or $P \leq 0.05$ was considered significantly heterogeneous. Publication bias was estimated by using funnel plots and Egger's test through the software STATA 14.0 (Stata Corporation, College Station, Texas). $P \leq 0.1$ was considered to indicate statistically significant publication bias.

Results

The selection of research objects and their characteristics

The processes of identifying and selecting studies are presented in Figure 1. Through the preliminary search, a total of 153 studies were screened out. By reading the titles and abstracts of these articles, the repetitive or irrelevant studies were excluded. After a detailed review of the remaining 16 studies that might meet the inclusion criteria, 5 articles with 781 patients were finally included in the meta-analysis. OS was documented in all studies. According to the NOS literature quality assessment scale, the quality score of all the studies was 7, indicating that the quality of the studies was relatively high (Table 1).

Association of PKM2 expression with OS

PKM2 has been reported as a key molecule in the metastasis of cancer, with it being over-expressed in various cancer tissues in comparison with paired normal adjacent tissue (NAT). However, there has been a lack of summary of different studies on PKM2 in ESCC that can provide the reader with extensive information on the clinical impact of PKM2 in ESCC. Consequently, the expression of PKM2 in ESCC was meta-analyzed by us. Eventually, the results showed that over-expression of PKM2 was associated with poor prognosis in patients with ESCC (HR=1.72, 95%CI: 1.41~2.09, $P \leq 0.01$) (Figure 2, A).

Association of PKM2 expression with clinicopathological features

PKM2 positivity and over-expression were significantly different between ESCC and its paired normal controls (OR=21.18, 95%CI: 6.46~69.47, $P \leq 0.01$) and significantly different between lymph node metastasis and non-metastasis (OR=2.38, 95%CI: 1.68~3.35, $P \leq 0.01$) (Figure 2, B and C). PKM2 positivity and over-expression were significantly associated with clinical stage I/II and clinical stage III/IV (OR=3.29, 95%CI: 2.27~4.77, $P \leq 0.01$) and significantly associated with T classification (OR=2.92, 95%CI: 2.05~4.16, $P \leq 0.01$) (Figure 2, D and E). But PKM2 positivity and over-expression were not significantly associated with differentiated degree (OR=1.40, 95%CI: 0.79~2.48, $P=0.25$) (Figure 2, F). Together, these data indicate that PKM2 over-expression could significantly promote lymph node metastasis, clinical stage and T classification in tissues of ESCC.

Heterogeneity analysis

Heterogeneity among the studies was analyzed by χ^2 test and I^2 test, and heterogeneity was found in correlation analysis of PKM2 expression between ESCC and NAT ($P \leq 0.05$, $I^2=86\%$) and degree of differentiation ($P \leq 0.05$, $I^2=63\%$). Therefore, the random effects model was used to analyze PKM2 expression between ESCC and NAT and differentiated degree, and fixed effects model was used for other correlation analysis.

Publication bias

Begg's funnel plots were performed to access the publication bias of the literatures. The sharps of the funnel plots did not reveal any evidence of obvious asymmetry (Figure 3). For the accuracy of the results, Egger's test was used to further evaluate publication bias and the results showed that there was no publication bias among the studies ($P > 0.05$, Table 2).

Discussion

It is currently believed that PKM2 plays an important role in glucose metabolism of malignant tumors, and PKM2 mediated Warburg effect can provide sufficient energy and a large number of metabolic intermediates for the rapid proliferation of tumor cells[16]. The reason is that under the regulation of various factors, PKM2 can switch between the highly active tetramer and the less active dimer[17]. The tetramer with pyruvate kinase activity, and the dimer with protein kinase activity[18]. The activity of PKM2 is regulated by a variety of post-translational modifications, such as phosphorylation, acetylation, sumoylation, hydroxylation, and oxidation, which prefer the formation of dimer PKM2 in tumor cells[18, 19]. PKM2 mutations can also change activity[20]. A study proved that PKM2 activity is also down-regulated by oxidation of C358 by ROS or hypoxia, leading to switching of the flux of glucose into the pentose phosphate pathway and glycolytic biosynthesis to generate NADPH for ROS detoxification and tumor progression[21]. Cytoplasmic PKM2 is a stable tetramer form, and nuclear PKM2 is a dimer form that plays the role of protein kinase and uses phosphoenolpyruvate (PEP) as a phosphate donor[22]. In the nucleus, STAT3 is phosphorylated at tyrosine 705 by PKM2.

The above studies suggest that PKM2 plays an important role in the occurrence and development of malignant tumors. However, the prognostic value of PKM2 in ESCC has not been determined. Therefore, the relationship between PKM2 expression and ESCC prognosis and clinicopathological parameters

was systematically evaluated and summarized through meta-analysis. The results showed that PKM2 was expressed differently in ESCC and paired NAT, and the prognostic analysis suggested that PKM2 over-expression was related to the poor prognosis of ESCC. It was also suggested that PKM2 over-expression promoted lymph node metastasis, clinical stage and T classification in ESCC tissues.

Similarly, some studies have suggested that PKM2 can be used as a prognostic marker for pancreatic ductal cell carcinoma (PDCA), breast cancer, hepatocellular carcinoma (HCC), and gallbladder carcinoma[23–25]. In addition, lymph node metastasis is the most important prognostic factor in ESCC[26], accurate nodal staging is crucial for ESCC[27]. Our study showed that PKM2 over-expression promoted lymph node metastasis of ESCC, suggesting that PKM2 may be a molecular target for lymph node metastasis of ESCC. Liu et al[28] reported that PKM2 inhibitor shikonin inhibited proliferation and glycolysis and induced cell apoptosis in HCC cells. James AD et al[29] reported that PKM2 inhibitor shikonin reduced PDAC cell proliferation, cell migration and induced cell death. Tang et al[30] reported that shikonin enhances sensitization of gefitinib against wild-type EGFR non-small cell lung cancer via inhibition PKM2/STAT3/cyclinD1 signal pathway. Another study reported that shikonin has a significant antitumor effect in the EC by regulating HIF1 α /PKM2 signal pathway[31]. Considering the complex function of PKM2 in cell biology, measures that inhibiting or silencing PKM2 possibly cause a wide range of effects in human body. Therefore, the therapeutic value of PKM2 should be systematically assessed.

However, a few limitations in our study should be admitted. Firstly, although we have not found any obvious evidences for publication bias from funnel plots and Egger tests, this meta-analysis was based on formally published articles with principally positive results. Hence, there is a potential publication bias that lowers the accuracy and validity of the results. Secondly, due to some relatively small sample studies and some missing information, the qualities of each included studies are not in the same level.

In summary, the present study suggests that PKM2 is crucial for the development of ESCC and PKM2 over-expression is associated with poor prognosis of ESCC and significantly promotes lymph node metastasis, clinical stage and T classification. PKM2 might be a potential prognostic biomarker and therapeutic target for ESCC.

Conclusions

The present study demonstrates that PKM2 plays a crucial role in ESCC. PKM2 level is significantly associated with ESCC prognosis and TNM staging. Additional research is needed to investigate how PKM2 promotes metastasis during ESCC carcinogenesis.

Abbreviations

ESCC

esophageal squamous cell carcinoma, ES = esophageal cancer, NAT = normal adjacent tissue, CI = confidence interval, HR = hazard ratio, OR = odds ratio, IHC = immunohistochemistry, NOS = Newcastle-Ottawa Scale, NA = not available, OS = overall survival, PK = Pyruvate kinase, PKM2 = pyruvate kinase M2, PEP = phosphoenolpyruvate, F = Female, M = Male, H = High, L = Low.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

Funding: The study was funded by the Natural Science Foundation of China (81960527, U1603284, 81860511), Key Research and Development Project of the Xinjiang Uygur Autonomous Region (2020B03003-1), Science and Technology Projects of Xinjiang Uygur Autonomous Region (2018E02067), TianshanXuesong Project of the Xinjiang Uygur Autonomous Region (2018XS19) and General Project from State Key Laboratory of Pathogenesis, Prevention, Treatment of High Incidence Diseases in Central Asia, Urumqi, Xinjiang Uygur Autonomous Region (SKL-HIDCA-2020-11,12).

Authors' contributions: QZ and SZ were responsible for literature screening, data extraction and quality evaluation. Disagreements were resolved by consensus or reviewed by LY. XL, QL and TL were responsible for concept, design, definition of intellectual content. QZ, AT and XH were responsible for statistical analysis. QZ was responsible for manuscript preparation, manuscript editing, and manuscript review. All authors read and approved the final manuscript. XL was designated as “corresponding author”.

Acknowledgements: Not applicable.

References

1. Yang X, Chen X, Zhuang M, Yuan Z, Nie S, Lu M, et al. Smoking and alcohol drinking in relation to the risk of esophageal squamous cell carcinoma: A population-based case-control study in China. *Scientific reports*. 2017;7(1):17249.
2. Doi T, Piha-Paul S, Jalal S, Saraf S, Lunceford J, Koshiji M, et al. Safety and Antitumor Activity of the Anti-Programmed Death-1 Antibody Pembrolizumab in Patients With Advanced Esophageal Carcinoma. *Journal of clinical oncology: official journal of the American Society of Clinical*

- Oncology. 2018;36(1):61–7.
3. Fu J, Xiong Z, Huang C, Li J, Yang W, Han Y, et al. Hyperactivity of the transcription factor Nrf2 causes metabolic reprogramming in mouse esophagus. *J Biol Chem*. 2019;294(1):327–40.
 4. Yao G, Yin J, Wang Q, Dong R, Lu J. Glypican-3 Enhances Reprogramming of Glucose Metabolism in Liver Cancer Cells. *BioMed research international* 2019; 2019: 2560650.
 5. Hsu M, Hung W. Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. *Mol Cancer*. 2018;17(1):35.
 6. Xu D, Liang J, Lin J, Yu C. PKM2: A Potential Regulator of Rheumatoid Arthritis via Glycolytic and Non-Glycolytic Pathways. *Frontiers in immunology*. 2019;10:2919.
 7. Sizemore S, Zhang M, Cho J, Sizemore G, Hurwitz B, Kaur B, et al. Pyruvate kinase M2 regulates homologous recombination-mediated DNA double-strand break repair. *Cell research*. 2018;28(11):1090–102.
 8. Li S, Huang P, Gan J, Ling X, Du X, Liao Y, et al. Dihydroartemisinin represses esophageal cancer glycolysis by down-regulating pyruvate kinase M2. *Eur J Pharmacol*. 2019;854:232–9.
 9. Liu Q, Liang M, Liu T, Vuitton L, Zheng S, Gao X, et al. M2 isoform of pyruvate kinase (PKM2) is upregulated in Kazakh's ESCC and promotes proliferation and migration of ESCC cells. *Tumour biology: the journal of the International Society for Oncodevelopmental Biology Medicine*. 2016;37(2):2665–72.
 10. Fukuda S, Miyata H, Miyazaki Y, Makino T, Takahashi T, Kurokawa Y, et al. Pyruvate Kinase M2 Modulates Esophageal Squamous Cell Carcinoma Chemotherapy Response by Regulating the Pentose Phosphate Pathway. *Annals of surgical oncology* 2015: S1461-8.
 11. Li W, Xu Z, Hong J, Xu Y. Expression patterns of three regulation enzymes in glycolysis in esophageal squamous cell carcinoma: association with survival. *Med Oncol (Northwood Lond Engl)*. 2014;31(9):118.
 12. Zhan C, Shi Y, Lu C, Wang Q. Pyruvate kinase M2 is highly correlated with the differentiation and the prognosis of esophageal squamous cell cancer. *Diseases of the esophagus: official journal of the International Society for Diseases of the Esophagus*. 2013;26(7):746–53.
 13. Zhang X, He C, He C, Chen B, Liu Y, Kong M, et al. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. *Pathol Res Pract*. 2013;209(8):510–5.
 14. Ma R, Liu Q, Zheng S, Liu T, Tan D, Lu X. PKM2-regulated STAT3 promotes esophageal squamous cell carcinoma progression via TGF- β 1-induced EMT. *Journal of cellular biochemistry* 2019.
 15. Liberati A, Altman D, Tetzlaff J, Mulrow C, Gøtzsche P, Ioannidis J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009;339:b2700.
 16. Li Z, Yang P, Li Z. The multifaceted regulation and functions of PKM2 in tumor progression. *Biochim Biophys Acta*. 2014;1846(2):285–96.
 17. van Niekerk G, Engelbrecht A. Role of PKM2 in directing the metabolic fate of glucose in cancer: a potential therapeutic target. *Cellular oncology (Dordrecht)*. 2018;41(4):343–51.
 18. Zahra K, Dey T, Ashish, Mishra S, Pandey U. Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. *Frontiers in oncology*. 2020;10:159.
 19. Wang Y, Liu J, Jin X, Zhang D, Li D, Hao F, et al. O-GlcNAcylation destabilizes the active tetrameric PKM2 to promote the Warburg effect. *Proc Natl Acad Sci USA*. 2017;114(52):13732–7.
 20. Liu V, Howell A, Hosios A, Li Z, Israelsen W, Vander Heiden M. Cancer-associated mutations in human pyruvate kinase M2 impair enzyme activity. *FEBS Lett*. 2020;594(4):646–64.
 21. Anastasiou D, Poulogiannis G, Asara J, Boxer M, Jiang J, Shen M, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science*. 2011;334(6060):1278–83.
 22. Xiaoyu H, Yiru Y, Shuisheng S, Keyan C, Zixing Y, Shanglin C, et al. The mTOR Pathway Regulates PKM2 to Affect Glycolysis in Esophageal Squamous Cell Carcinoma. *Technol Cancer Res Treat*. 2018;17:1533033818780063.
 23. Hu H, Tu W, Chen Y, Zhu M, Jin H, Huang T, et al. The combination of PKM2 overexpression and M2 macrophages infiltration confers a poor prognosis for PDAC patients. *J Cancer*. 2020;11(8):2022–31.
 24. Yang Y, Wu K, Liu Y, Shi L, Tao K, Wang G. Prognostic significance of metabolic enzyme pyruvate kinase M2 in breast cancer: A meta-analysis. *Medicine*. 2017;96(46):e8690.
 25. Zhu H, Luo H, Zhu X, Hu X, Zheng L, Zhu X. Pyruvate kinase M2 (PKM2) expression correlates with prognosis in solid cancers: a meta-analysis. *Oncotarget*. 2017;8(1):1628–40.
 26. Wu L, Yang X, Cao W, Zhao K, Li W, Ye W, et al. Multiple Level CT Radiomics Features Preoperatively Predict Lymph Node Metastasis in Esophageal Cancer: A Multicentre Retrospective Study. *Frontiers in oncology*. 2019;9:1548.
 27. Li B, Li N, Liu S, Li Y, Qian B, Zhang Y, et al. Does [18F] fluorodeoxyglucose-positron emission tomography/computed tomography have a role in cervical nodal staging for esophageal squamous cell carcinoma? *J Thorac Cardiovasc Surg*. 2020;160(2):544–50.
 28. Liu T, Li S, Wu L, Yu Q, Li J, Feng J, et al. Experimental Study of Hepatocellular Carcinoma Treatment by Shikonin Through Regulating PKM2. *Journal of hepatocellular carcinoma*. 2020;7:19–31.

29. James A, Richardson D, Oh I, Sritangos P, Attard T, Barrett L, et al. Cutting off the fuel supply to calcium pumps in pancreatic cancer cells: role of pyruvate kinase-M2 (PKM2). *British journal of cancer*. 2020;122(2):266–78.
30. Tang J, Ren Y, Zhao J, Long F, Chen J, Jiang Z. Shikonin enhances sensitization of gefitinib against wild-type EGFR non-small cell lung cancer via inhibition PKM2/stat3/cyclinD1 signal pathway. *Life sciences*. 2018;204:71–7.
31. Tang J, Zhao J, Long F, Chen J, Mu B, Jiang Z, et al. in vitro Efficacy of Shikonin against Esophageal Cancer Cells and its possible mechanisms and. *J Cancer*. 2018;9(1):32–40.

Tables

Table 1
Main characteristics of the 5 included studies in the meta-analysis

Study year	Country	Technology	Sample size	Age Medium	Gender (F/M)	PKM2(H/L)	Follow-up (months)	Outcome	HR(95%CI)	Cut-off value	NOS score
Fukuda2015 ^[10]	Japan	IHC	205	NA	30/175	104/101	47.9 ± 43.4	OS	1.850(1.200–2.780)	Score ≥ 6	7
Li2014 ^[11]	China	IHC	141	60	54/87	59/82	NA	OS	1.214(0.728–2.026)	Score ≥ 0.75	7
Zhan2013 ^[12]	China	IHC	210	NA	48/162	167/43	overall 72.0	OS	1.748(1.277–2.395)	Score ≥ 4	7
Zhang2013 ^[13]	China	IHC	86	65(41–81)	22/64	62/24	NA	OS	2.358(1.156–4.812)	Score ≥ 4	7
Ma2019 ^[14]	China	IHC	139	NA	32/107	103/36	NA	OS	1.754(1.070–2.876)	Score ≥ 3	7

Abbreviations: CI=confidence interval, HR=hazard ratio, OR=odds ratio, IHC=immunohistochemistry, NOS= Newcastle-Ottawa Scale, NA=not available, OS=overall survival, PKM2=pyruvate kinase M2, F=Female, M=Male, H=High, L=Low.

Table 2
Results of Egger's test

Comparison	<i>t</i>	<i>P</i> -value	95%CI
ESCC and NAT	1.65	0.197	2.838 to 8.966
Lymph node metastasis	2.20	0.115	1.206 to 6.622
Clinical stage	0.26	0.820	10.255 to 11.571
T classification	0.38	0.740	16.753 to 20.005
Differentiated degree	1.47	0.237	18.106 to 6.649

Abbreviations: ESCC=esophageal squamous cell carcinoma, NAT=normal adjacent tissue, CI=confidence interval.

Figures

Figure 1

Flow diagram of study selection process and specific reasons for exclusion in this meta-analysis.

Figure 2

Difference for the association of PKM2 in ESCC. (A) Forest plot of overall association between PKM2 over-expression and OS in ESCC (fixed effects). (B) Forest plot of the association between PKM2 expression in ESCC and paired NAT (random effects). (C) Forest plot of the association between PKM2 expression in lymph node metastasis and non-metastasis tissues (fixed effects). (D) Forest plot of the association of PKM2 expression in clinical stage (fixed effects). (E) Forest plot of the association of PKM2 expression in T classification of ESCC (fixed effects). (F) Forest plot of the association of PKM2 expression in differentiated degree of ESCC (random effects). The square and the horizontal lines correspond to the study-specific OR/HR and 95% CI. The diamond represents the summary OR/HR and 95% CI.

Figure 3

Funnel plots for publication bias in an association analysis of PKM2 expression among different clinicopathological parameters. (A) Funnel plot of overall association between PKM2 over-expression and OS in ESCC. (B) Funnel plot of the association between PKM2 expression in ESCC and paired NAT. (C) Funnel plot of the association between PKM2 expression in lymph node metastasis and non-metastasis tissues. (D) Funnel plot of the association of PKM2 expression in clinical stage. (E) Funnel plot of the association of PKM2 expression in T classification of ESCC. (F) Funnel plot of the association of PKM2 expression in differentiated degree of ESCC.