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Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-21-442

Reviewer A

This is a meta-analysis aimed at investigating the significance of pyruvate kinase 2 (PKM2) expression in esophageal squamous cell carcinoma. Based on the analysis of 5 studies, the authors concluded high PKM2 expression was associated with a worse overall survival and correlated with lymph node metastases, clinical stage and T-stage. While the data are interesting, there are some revisions needed in order better support the conclusions. The following revisions are suggested:

- In the background, please discuss how "increased" nuclear expression was determined in the Sizemore paper.

Reply: This is a constructive suggestion. We have modified our text as advised (see Page 5, line 95-98) .

Changes in the text: Sizemore et al. (7) confirmed that the serine/threonine kinase ataxia telangiectasia–mutated (ATM) phosphorylates nuclear PKM2 at T328 following DNA damage, leading to the accumulation of PKM2 in the nucleus.

- Section 3.1, change objects to subjects.

Reply: We appreciate the reviewer for his or her great carefulness in going through our manuscript. At the same time, we consider that "participants" may be more appropriate than "subjects", so "objects" was replaced by "participants" in our text. We have modified our text as advised (see Page 7, line 155).

Changes in the text: 3.1 The selection of research participants and their characteristics.

- Sections 3.2 and 3.3, please include more detail about the PKM2 expression analysis from the 5 papers. Did all use the same antibody, protocol, scoring system, etc. All of the results hang on the expression of PKM2, yet there is no description of this very important aspect of the analysis.

Reply: Thanks for the reviewer's critique that will make our paper stronger than before. We have modified our text as advised (see Page 8, line 170-177) and summarized it in Supplementary Table 1 (shown below).

Changes in the text: Among the 5 included articles, different antibody manufacturers were used, and the dilution ratio was 1:100, except in the study of Zhang et al. (13), in which the ratio was 1:30. The IHC method used was either the EnVision (Agilent Technologies, Santa Clara, CA, USA) method or the streptavidin peroxidase (SP) method. The scoring systems mainly included staining intensity and the percentage of positive cells. The staining intensity of 5 articles was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was scored slightly differently between the 5 articles (Supplementary Table 1).

Supplementary Table 1. PKM2 expression analysis from the 5 papers

Studies	Antibody	Protocol	Scoring system
Fukuda2015[10]	Proteintech Group, Campbell, USA（1:100）	EnVision	Intensity: 0, negative; 1, weak; 2, moderate; 3, strong. Percentage of positive cells: 0, negative; 1, 1 - 30 % positive cells; 2, 31 - 60 % positive cells; 3, 61 - 80 % positive cells; 4, 81 - 100 % positive cells. The score = staining intensity × percentage of cells stained. They qualified PKM2 expression as “weak” when the score was less than 6 and “strong” when it was higher than 6.
Li2014[11]	EPR10138(B), EPITOMICS, California, US （1:100）	Streptavidin peroxidase (SP)	Intensity: negative, 0; weak, 1+; moderate, 2+; intense, 3+. A mean percentage of positive tumor cells was determined in five areas at X400 magnifications and assigned from 0 to 100 %. Thus, the percentage of positive tumor cells and the staining intensity were multiplied to produce a weighted score for each case: ranging from 0 (0 of cells staining) to 3 (100 % of the cells staining at 3+ intensity). For convenience in statistical analysis, they defifined the score<0.75 as low expression and ≧0.75 as overexpression.
Zhan2013[12]	Cell Signaling Technology, Boston, MA, USA （1:100）	EnVision	Staining intensity was scored as: 1, weak; 2, moderate, and 3, intensive. The percentage of positive cells was rated as follows: 1, 1–10% positive cells; 2, 11–50%; 3, 51–80%; and 4, more than 80% positive cells. Scores for percentage of positive cells and for expression intensities were multiplied to calculate an immunoreactive score (IRS). Finally, They separated the specimens according to the PKM2 protein level in four groups: negative, IRS 0–1; weak, IRS 2–4; moderate, IRS 6–8; strong, IRS 9–12.
Zhang2013[13]	Abgent, CA, USA （1:30）	Streptavidin peroxidase (SP)	Staining intensity: 0 score for non-staining, 1 score for poor staining, 2 scores for moderate staining, and 3 scores for strong staining. The percentage of positive cells (P) was scored on a scale of 0–3: 0 score for non-staining, 1 score for <20%, 2 scores for 20% to <75%, and 3 scores for ≥75%. The total histological score (H) was calculated by the formula: H = I × P (score <4 for low expression group, score ≥4 for high expression group).

Ma2019[14]	**Proteintech, Chicago (1:100)**	**EnVision**	**Four grades each were assigned for staining intensity (0, none; 1, weak; 2, moderate; and 3, strong) and percentage of positive cells (0, <10%; 1, 10%-25%; 2, 25%-50%; and 3, >50%). ESCC patients were classified into two groups according to total score (staining intensity plus positive cell score), specifically, "low expression" (total score, 0-2) and "high expression" (total score, 3-6) for analysis of prognosis between groups.**

- Section 3.3, change "differentiated degree" to "tumor differentiation".

Reply: This is a good question with which we respectfully agreed. We have modified our text as advised (see Page 9, line 198-199, 205 and 207).

Changes in the text: (1) However, PKM2 positivity and overexpression were not significantly associated with **tumor differentiation** (OR = 1.40, 95% CI: 0.79-2.48; $P = 0.25$; Fig. 2F). (2) 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 ($P < 0.05$; $I^2 = 86\%$) and **tumor differentiation** ($P < 0.05$; $I^2 = 63\%$). Therefore, the random effects model was used to analyze PKM2 expression between ESCC and NAT and **tumor differentiation**, and the fixed effects model was used for other correlation analyses.

- Section 3.3, while there is no data presented in this manuscript that supports the interpretation that PKM2 over-expression "significantly promotes" lymph node metastases; a better choice of would might be that it "correlates with". This statement and the use of the word "promote" should be modified throughout the discussion as well.

Reply: This is a good question with which we respectfully agreed. We have modified our text as advised (see Page 3, line 64; Page 9, line 200; Page 11, line 242 and 254; Page 12, line 284).

Changes in the text: (1) High PKM2 expression denotes worse OS in ESCC patients, and **correlates with** the lymph node metastasis, clinical stage, and T classification. (2) Together, these data indicate that PKM2 overexpression could significantly **correlates with** lymph node metastasis, clinical stage, and T classification in tissues of ESCC. (3) It was also suggested that PKM2 overexpression **correlates with** lymph node metastasis, clinical stage, and T classification in ESCC tissues. (4) Our study showed that PKM2 overexpression **correlates with** lymph node metastasis of ESCC, suggesting that PKM2 may be a molecular target for lymph node metastasis of ESCC. (5) In summary, the present study suggests that PKM2 is crucial for the development of ESCC and that PKM2 overexpression is associated with poor prognosis of ESCC and **correlates with** lymph node metastasis, clinical stage, and T classification.

- Table 1:

* Age "medium" should be "median"

* For Cut-off value, please describe what this means. Is this the number used to determine high vs low expression? If so, the range is very high.
* Include in the table what each study used to conclude low vs high expression

Reply:

1. We were impressed by the carefulness of the reviewer. We have modified our text as advised (see Page 20, line 462).

Changes in the text: See Table 1 below.

2. Excellent question that we appreciate. We have modified our text as advised (see Page 8, line 178-186).

Changes in the text: Therefore, the definition of the cutoff value in the 5 articles was different. For Fukuda t al. (10), Zhan et al. (12), and Zhang et al. (13), the cutoff value was defined as the median value multiplied by the intensity score and the percentage of positive cells score. For Ma et al. (14), the cutoff value was the median of the staining intensity score plus the percentage of positive cells score. For Li et al. (11), the cutoff value was defined by combining the weighted score generated by the multiplication of the intensity score and the percentage of positive cells score and statistical analysis. All of them qualified PKM2 expression as "low" when the immunoreactive score (IRS) was less than the cutoff value and "high" when it was higher than the cutoff value.

3. We have come to realize the point. We have modified our text as advised (see Page 20, line 462).

Changes in the text: See Table 1 below.

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

dy year	Country	Technology	Sample size	Age Median	Gender (F/M)	PKM2 (L/H)	Follow-up (months)	Outcome	HR (95%CI)	Cutoff value	NC sco
kuda2015[10]	Japan	IHC	205	NA	30/175	101/104	47.9±43.4	OS	1.850(1.200-2.780)	Score≧6	7
2014[11]	China	IHC	141	60	54/87	82/59	NA	OS	1.214(0.728-2.026)	Score≧0.75	7
an2013[12]	China	IHC	210	NA	48/162	43/167	overall 72.0	OS	1.748(1.277-2.395)	Score≧4	7
ang2013[13]	China	IHC	86	65(41-81)	22/64	24/62	NA	OS	2.358(1.156-4.812)	Score≧4	7
2019[14]	China	IHC	139	NA	32/107	36/103	NA	OS	1.754(1.070-2.876)	Score≧3	7

- Table 2: Differentiated degree should be called Tumor differentiation

Reply: Yes, we agree with the reviewer. We have modified our text as advised (see Page 21, line 479).

Changes in the text: See Table 2 below.

Table 2. Results of Egger's test

Comparison	*t*	*P*-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**
Tumor differentiation	**1.47**	**0.237**	**18.106 to 6.649**

- Figure 2: Please explain why panels D and E only have 4 papers included in the analysis.
Reply: For panels D, Ma et al. 's paper missing the specific number of clinical stage cases; for panels E, Zhang et al. 's paper missing the specific number of T classification cases. Therefore, only 4 papers were included in panels D and E.

Minor:
- Significant grammatical and typographical corrections throughout the manuscript need to be corrected. A few examples include, but are not limited to...
* literatures should be literature
* evidences should be evidence
* qualities should be quality
Reply: Yes, we have came to realize the point and significant grammatical throughout the manuscript has been revised and marked in the manuscript.

Reviewer B

This manuscript discusses the role of PKM2 on ESCC and confirmed PKM2 level is significantly associated with ESCC prognosis and TNM staging. Although this review has interesting aspect, there are several critical issues before publication.
The significance and new findings of this meta-analysis are not clear.
Reply: Thanks for the reviewer's critique that will make our paper stronger than before. We have modified our text as advised (see Page 11, line 244-258) .
Changes in the text: Similarly, some studies have suggested that PKM2 can be used as a prognostic marker for pancreatic ductal adenocarcinoma (PDAC), breast cancer, hepatocellular carcinoma (HCC), and gallbladder carcinoma (23-25). However, the prognostic value of PKM2 remains controversial. We performed this meta-analysis to provide a more comprehensive and direct understanding of whether PKM2 can be used as a prognostic marker for ESCC. In addition, as lymph node metastasis is the most important prognostic factor in ESCC (26), accurate nodal staging is crucial for the treatment of ESCC (27). Some studies report PKM2 expression to not be associated with lymph node metastasis in ESCC (10,12,13). Therefore, a meta-analysis combining

the results of several studies enabled a more comprehensive overview. Our study showed that PKM2 overexpression correlates with lymph node metastasis of ESCC, suggesting that PKM2 may be a molecular target for lymph node metastasis of ESCC. It is also controversial whether PKM2 is associated with tumor differentiation in ESCC. Our results showed that PKM2 was not associated with tumor differentiation.

The authors did not mention NAC (reference 10 contains NAC cases).

Reply: The point is well taken. We have modified our text as advised (see Page 11 and 12, line 259-274) .

Changes in the text: An interesting finding was that strong PKM2 expression significantly correlated with poor response to chemotherapy. Fukuda et al. (10) showed that strong PKM2 expression significantly correlated with decreased OS in patients who received neoadjuvant chemotherapy followed by surgery, and PKM2 expression was not affected by the neoadjuvant chemotherapy. Therefore, the therapeutic value of PKM2 should be systematically assessed. Liu et al. (28) reported that the PKM2 inhibitor shikonin inhibited proliferation and glycolysis and induced cell apoptosis in HCC cells. James 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 epidermal growth factor receptor (EGFR) non-small cell lung cancer via inhibition of the PKM2/STAT3/cyclinD1 signal pathway. Another study reported that shikonin has a significant antitumor effect in EC by regulating the HIF1α/PKM2 signal pathway (31). Considering the complex function of PKM2 in cell biology, measures that inhibit or silence PKM2 possibly cause a wide range of effects in the human body, especially in patients who are chemotherapy resistant.

Evaluation of immunohistochemistry is not sufficient.

Reply: Thanks for the reviewer's critique that will make our paper stronger than before. We have modified our text as advised (see Page 8, line 170-177) and summarized it in Supplementary Table 1 (shown below).

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			patients were classified into two groups according to total score (staining intensity plus positive cell score), specifically, "low expression" (total score, 0-2) and "high expression" (total score, 3-6) for analysis of prognosis between groups.