

Peer Review File

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Reviewer A

Comment 1: As prognostic study, authors should follow and fill the report of REMARK guidelines - <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3362085/>

Reply 1: We think this is an excellent suggestion. We made some changes following the REMARK guidelines and attached the checklist (see the attached REMARK checklist).

Changes in the text: the REMARK checklist

Comment 2: Include MeSH terms in your keywords

Reply 2: We added some additional MeSH terms in the keywords (see Page 2, line 39-40).

Changes in the text: Keywords: CKS2, oral squamous cell carcinoma, nomograms, prognosis, immunity, cell proliferation

Comment 3: Concerning nomogram model, please consider the application of information coming from this study "Development and validation of prognostic models for oral squamous cell carcinoma: A systematic review and appraisal of the literature"

Reply 3: Thanks for your suggestion. We applicated this valuable article (see Page 13, line 415).

Changes in the text: However, there were methodological differences in model development and validation(47).

Comment 4: In the abstract "inferior clinical outcomes" is not a good way to express poor outcomes

Reply 4: We have modified our this sentence according to your suggestions (see Page 2, line 32).

Changes in the text: Our findings indicated a significant upregulation of CKS2 expression in OSCC tissues compared to normal groups, which was positively associated with poor clinical outcomes.

Comment 5: Correct CSK2 capital letters along the whole manuscript

Reply 5: We are very sorry for our incorrect writing. These misspellings have been corrected (see Page 3, line 53 and Page 12, line 382).

Changes in the text: CKS2

Comment 6: Include ethical approval code

Reply 6: we have added the ethical approval code as advised (see Page 14, line 437).

Changes in the text: The ethics committee at the Hospital of Stomatology, Sun YatSen University granted approval for this study (Ethics approval Code: KQEC-2022-15-01).

Comment 7: Include detailed inclusion/exclusion criteria. For example TCGA content of HPV+ tumors might lead to biased results as HPV should be considered differently from HPV- tumors

Reply 7: We added the detailed inclusion criteria (see Page 4-5, line 93-95).

Changes in the text: Patients who met the following criteria were included in the study: (1) histologically verified primary OSCC; (2) sample size in the dataset was more than 70; (3) patients with complete RNA-seq data and survival data. The OSCC samples

with complete clinical data in TCGA-OSCC and GSE4743 datasets were included in the development of the Nomogram.

Comment 8: Move this sentence to statistical paragraph: All these data were included in our study, and we utilized R software (version 4.2.2, 98 <https://www.Rproject.org>) for data analysis

Reply 8: we have modified this part as advised (see Page 5, line 101-102 and Page 8, line 221-228).

Changes in the text: 2.16 Statistical Analysis

R software (version 4.2.2, <https://www.Rproject.org>) and GraphPad Prism 9.0 software were used for statistical analysis in this study. Wilcoxon test was employed to compare the expression of CKS2 among the two groups. Correlation assessment was conducted by calculating Pearson's r-value. Mann–Whitney U, Kruskal–Wallis, and Chi-square tests were used to assess the association between CKS2 expression and clinicopathological parameter. One way ANOVA and student's t test were utilized to compare the control group and the experimental group. A p value of 0.05 or less was considered statistically significant.

Comment 9: Delete this sentence: which is highly cited and valuable.

Reply 9: We have deleted this sentence (see Page 5, line 108).

Changes in the text: The GEPIA2 web server (<http://gepia2.cancer-pku.cn/>) was used to analyse gene expression in tumor and normal samples obtained from the TCGA and GTEx databases.

Comment 10: This is unclear: Using the GEPIA2, a study was conducted on 33 cancer subtypes to analyze the relationship between differential expression of CKS2 and the prognosis of OSCC patients

Reply 10: We have rewritten this sentence to make it more fluent and clear (see Page 5, line 110-113).

Changes in the text: We performed differential expression analysis and OS analysis of CKS2 by the GEPIA2 on the 33 cancer subtypes in the TCGA pan-cancer datasets.

Comment 11: Why did you test CSK2 among all 33 cancer subtypes? You should limit analysis to OSCC TCGA

Reply 11: Given the role of CKS2 in multiple cancers reported in the literature, we hope that a pan-cancer analysis will help illustrate the importance of CKS2 in tumors. Consequently, this elucidates our motivation to investigate the role of CKS2 specifically in OSCC. However, we are also flexible if the reviewers or the editor insists on removing them from the section.

Comment 12: Clearly report OR - HR 95% c.i. and p-value when reporting results

Reply 12: We have made correction according to the Reviewer's comments (see Page 9, line 264; Page 10, line 295-297,301; Page 11, line 317-333).

Comment 13: Image quality is very bad. I believe it's submission system, better adding more supplemental figures instead of merging in one smaller panel

Reply 13: Thank you for your suggestion. In addition to the requirements in the submission system, we have uploaded high quality vector images in the supplemental files.

Comment 14: Why results from WGCN analysis were not validated in the cell culture models?

Reply 14: We sincerely appreciate the valuable comments. We agree that more study would be useful to support the results from WGCNA. Unfortunately, due to the limited time and funding, we are currently unable to further supplement this experiment. In this study, we aimed to preliminarily explore the function of CKS2 in OSCC and to analyze whether it might serve as a prognostic biomarker. In follow-up research, we intend to verify our analytical findings using sophisticated tools like flow cytometry and further investigate the molecular pathways.

Reviewer B

Comment: in paragraph 3.7 the mentioned figure is Fig.6 and not Fig.8.

Reply: We feel sorry for our carelessness. In our resubmitted manuscript, the figure name is revised. Thanks for your correction.

Changes in the text: (Figure 6).