

Peer Review File

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Reviewer A

Comment 1: There are several mentions throughout the text of "head and neck cancer" and "HNSCC". Even though the latter is part of the former, the authors must review whether all the data presented here refer only to HNSCC. This sounded confusing to me a few times while reading.

Reply 1: Thank you very much for your professional comments. Firstly, we apologies for the confused expression in our manuscript. We corrected the mentions of "head and neck cancer" into "HNSCC" throughout the manuscript.

Changes in the text: Line 82, 184-185, 314.

Comment 2: The results show that S100 is implicated beyond prognosis. I suggest: "An integrated bioinformatic analysis of the S100 in head and neck squamous cell carcinoma".

Reply 2: Thank you for your suggestion. We changed the title into "An integrated bioinformatic analysis of the S100 in head and neck squamous cell carcinoma".

Changes in the text: We rewrote the title. See Line 1-3 and Line 30-32.

Comment 3: The authors should check the English language used throughout this paper to ensure that it is correct, clear, and concise. There are errors in incoherence, typography, and punctuation that should be revised.

Reply 3: Thank you for your valuable and thoughtful comments. We have carefully checked and improved the English writing in our revised version.

Changes in the text: We have made corrections throughout the manuscript.

Comment 4: Lines 57-60: Start the introduction by talking about head and neck cancers and only then mention the importance of HNSCC. The way presented seems confusing to me.

Reply 4: Thank you. We have corrected the lines in the manuscript as advised.

Changes in the text: We changed the order of Lines 59-63 and related citation.

Comment 5: A better approach to the role of S100 in the tumor microenvironment of HNSCC might be valuable. I am also missing a citation regarding the role of S100 proteins in tumor immunity.

Reply 5: Thanks for your comment. It is really important to present the role of S100 proteins in tumor immunity. We added citations of the role of S100 proteins in tumor immunity.

Changes in the text: See Page 3, Line 74-76.

Comment 6: Standardize the section titles: they should either present the results or be the summarized results. The first title sounds strange to me.

Reply 6: Thanks for your professional suggestion. We standardized the section

title. And the new title for the first section should be “Atlas of selected 19 S100 protein members in HNSCC”.

Changes in the text: We rewrote the title in Line 174-175.

Comment 7: The results section sounds like a discussion. The results should present the findings without personal impressions of the authors. Transpose lines 205-207 for the Discussion. EMT section would benefit from this same care.

Reply 7: Thank you for your suggestion. We changed the content in Results section and Discussion section accordingly.

Changes in the text: We deleted Lines 206-207, 215-217, 229-232.

Comment 8: The discussion is exhaustive and could be revised to discuss more didactically the main result of the work: “S100 family members are implicated in the initiation, progression, metastasis, and survival of head and neck squamous cell carcinoma”. Lines 274-361: A 4-page paragraph is not normal for me.

Reply 8: Thank you for your comment. We deleted exhaustive Lines and revised related content.

Changes in the text: See in Line 274-415.

Comment 9: Did the work have no limitations?

Reply 9: Thank you for your comment. Our study had several limitations which could not be ignored. Firstly, our results were based on the bioinformatics analysis of the TCGA data and were not validated in clinical patient cohort or other public databases. Secondly, our study was retrospective research and a prospective study needs to be implemented to verify the findings in the future. Thirdly, some lack of clinical parameters in the datasets may decrease the statistical validity and reliability

Changes in the text: We added this part of content in our discussion section.

Comment 10: Why will this work be important for other researcher in the future? Why are these data important? Can they impact the treatment of HNSCC? This should be better addressed in the discussion.

Reply 10: Our study provides innovative insights into the roles of S100 family during HNSCC development and progression and established a promising prognostic model to evaluate patient prognosis. This could be a hint for those who want to focus on this group of molecules in HNSCC progression.

Changes in the text: We added this part of content in our discussion section.

Comment 11: Also in the discussion, I miss a more detailed argumentation of the contrasting findings of the GO x association of the mRNA expression status of the eight-gene prognostic signature with tumor-infiltrating immune cells, and its impact on the HNSCC.

Reply 11: Thank you. We added a more detailed discussion about the contrasting findings of the GO x association of the mRNA expression status of the eight-gene

prognostic signature with tumor-infiltrating immune cells, and its impact on the HNSCC in our manuscript.

Changes in the text: see Line 280-288.

Comment 12: In figure 1A, the words seem too small. Wouldn't there be another way to better present this data?

Reply 12: Thanks for informing us of this.

Changes in the text: We revised the figure with larger words.

Comment 13: Figure 6 is great! Congratulations!

Reply 13: Thank you for your comment.

Reviewer B

In the manuscript entitled " An integrated bioinformatic analysis of the S100 gene family for the prognosis of head and neck squamous cell carcinoma" the authors investigated, in silico, the possible role of S100 gene family/pathway in head and neck tumorigenesis.

The work has interesting results on the interaction between 100 and head and neck cancer. I believe that some lack of information and/or methodological impair the findings.

Comment 1: Abstract: Please review the journal's guidelines in full. In the abstract (lines 32-34) I suggest removing the brief introduction on S100, as it is in the area of study purposes, or adapting it as appropriate into an introductory topic. The methodology lacks information such as DEG analysis, road enrichment, among others.

-Reply: Thanks for informing us of this.

-Changes in the text: We have deleted Line 34-36 in the manuscript as advised. And in the part of methodology, we added the methods for DEG analysis in Line 41.

Comment 2: Still about the abstract (line 50 and 54) I didn't understand how it was possible to say about the onset of the disease.

-Reply: We reached the conclusion from the results as shown in the table 1.

Comment 3: The authors mentioned in the introduction (line 75) that previous studies reported associations of S100 with EMT and CSCs in other types of carcinomas. There was a demonstration in the present study between interaction with EMT, however it was not demonstrated with CSCs. I believe this information is important, taking into account that there are packages in R that do this analysis (TCGABiolinks

- https://bioconductor.org/packages/release/bioc/vignettes/TCGABiolinks/inst/doc/stemness_score.R)

Reply: Thank you for your comment.

Changes in the text: We added the citation about reported associations of S100 with CSCs, see Line 78, and we added the figure for associations of S100 with CSCs,

see Line 264 and Figure S5.

Comment 4: Is this study sufficiently powered to drive unequivocal conclusions? Please present the results of the power analyses demonstrating that high and low expression are sufficient to power this study.

Reply: Thank you for your comment. We feel sorry for we cannot present related analysis now. We have consoled our technical personnel, however, there was no reply by the time we resubmit the revised manuscript.

Comment 5: How was the TCGA data extraction performed (line 92-93) using R? Direct website?

Reply: Thanks for your comment.

-Changes in the text: We have revised related content in Line 100-101.

Comment 6: At the time of the analysis between tumor tissue and normal tissue, were any criteria used (line 95)? Only matched patients? Just primary tumors? What values were taken into account?

Reply: Thanks for your comment. We include all patients in the analysis.

Comment 7: Were any genomic signatures taken into account for EMT (line 99)? Please cite the article and place the respective genes.

-Reply: Thanks for your comment.

-Changes in the text: We have added the five genes with citation related to EMT. See Line104-106.

Comment 8: Please cite the articles of the respective packages used. For example ggstasplot (line 100).

-Reply: Thanks for your comment.

-Changes in the text: We have added the citation for the packages we used. See line 104-105.

Comment 9: Has the pathway enrichment analysis not taken into account the FDR value (line 106-107)?

-Reply: Thank you for your comment.

-Changes in the text: We have added the threshold of FDA value for GO analysis. See line 113-115.

Comment 10: Please mention the average of the values of low and high expressions (line 118-119)

Reply: T Thank you for your comment.

-Changes in the text: We added the table S2 and related content Line 222-223 in the text.

Comment 11: Was the value of 501 patients with HNSCC mentioned in line 122-

123 the same used throughout the research? Why did you choose 501, what were the criteria?

Reply: Thank you for your comment. We exclude those without clinical information.

Comment 12: I didn't understand line 147: "after initial treatment versus S100s expression (low/high) were evaluated." Against what?

Reply: Thank you for your comment.

-Changes in the text: We have revised lines 154-155.

Comment 13: A data availability (line 148 -156) section is not required for everyone as they are present in the respective topics.

Reply: Thank you for your comment.

-Changes in the text: We have deleted Line 157-165.

Comment 14: Has the genomic signature suggested in the article been validated in any other bank?

Reply: Thank you for your comment. The genomic signature suggested in the article has not been validated in other bank.

Comment 15: Figure 6 is cropped. Please add a new image! Also, I suggest resolution on all images.

Reply: We will upload the figure again in the submission system.