



“A Grant-in-Aid Institute under Department of Atomic Energy, Govt. of India”

February, 21 2023

To,
Editor In Chief,
WJSC

Sub: Answers to Reviewer comments

Dear Sir,

Thank you for invitation to submit a review article. We are submitting our revised manuscript entitled **Molecular Signalling in Cancer stem cells of tongue squamous cell carcinoma: therapeutic implications and challenges** as an invited review article for publication in your esteemed journal.

We have gone through the Comments of the Reviewer and have addressed them point wise that is as follows:

Reviewer 1:

- 1. Does CD44v8 exist? (page 4 line 7) Reference No.6 describes CD44v8-10, which is a variant containing three consecutive exons v8, v9, and v10**

We thank reviewer for the comment. The name of the variant is corrected as ‘CD44v8-10’

- 2. References should be correctly listed. Papers that should be described with Article ID are not described as such. Examples: References Nos. 27, 28, 32, 33, 34, 38, 41, 43, 52, 55, 76, and 85. Reference No. 7 does not include the name of the journal. The doi of reference No. 9 is incomplete. Reference No. 69 has no page numbers or article ID.**

We thank reviewer for the comment. The PMIDs and PMCIDs of the references 27, 28, 32, 33, 34, 38, 41, 43, 52, 55, 76, and 85. The name of the journal has been added to reference no. 7. The doi of reference no. 9 is added. Page number as well as article ID are added for reference no. 69.

- 3. The figures are all too general. It should relate to the events observed in tongue squamous cell carcinoma (TSCC), and the diagram should be made so that the role of each signal pathway in TSCC can be understood.**



We thank reviewer for the comment. Following changes have been made to the figures keeping relevance to the references included in the article:

Figure 1: The ‘Wnt on’ section of the figure shows the downregulation of the pathway by miRNAs listed in the review viz. miR-29a, miR-638 and miR-106a*. Further, the figure includes downregulation of Wnt pathway by Quercetin and Niclosamide. The figure shows binding of SOX8 to the promoter of Frizzled-7 and inducing its expression

Figure 2: The ‘Hedgehog on’ section of the figure shows increase in angiogenesis and invasiveness on activation of pathway. Further, it shows increase in spheroid formation ability with upregulation of GLI1 as well as downregulation of stem cell markers like CD44, OCT-4 and BMI-1 with decrease in GLI3 reexpression.

Figure 3: The figure shows decrease of spheroid formation ability and increase in chemosensitization with decrease in NOTCH1 expression as well as increase in expression of stemness markers like CD44, OCT4 and SOX2 on increase in NOTCH4 expression. Further, the figure shows increase in spheroid forming ability and increase in expression stemness markers (SOX2, CD44 and OCT4) on activation of Notch intracellular domain (NICD). The figure also includes increased invasiveness on activation of the pathway.

Figure 4: The figure shows the increase in metastasis and invasiveness relevant to the findings of the reports referenced in the review. The figure also shows increase in c-MET in cells with high Aldehyde dehydrogenase (ALDH) activity.

Reviewer 2:

1. I kindly ask to complete the discussion considering the role of PD L1 in this context with the important questions/implications related to the progression from dysplasia to carcinoma and the management of the specimens as well as the assesment by the pathologist, given the important effects on prognosis and therapy. In this regard quote:
- Atlas of PD-L1 for Pathologists: Indications, Scores, Diagnostic Platforms and Reporting Systems. *J Pers Med.* 2022 Jun 29;12(7):1073. doi: 10.3390/jpm12071073. PMID: 35887569 - PD-L1 in oral squamous cell carcinoma: A key biomarker from the laboratory to the bedside. *Clin Exp Dent Res.* 2022 Jun;8(3):690-698. doi: 10.1002/cre2.590. Epub 2022 May 20. PMID: 35593124 - PD-L1 evaluation in head and neck squamous cell carcinoma: Insights regarding specimens, heterogeneity and therapy. *Pathol Res Pract.* 2021 Oct;226:153605. doi: 10.1016/j.prp.2021.153605. Epub 2021 Sep 1. PMID: 34530257 - Prevalence of PD-L1 expression in head and neck squamous precancerous lesions: a systematic review and meta-analysis. *Head Neck.* 2020 Oct;42(10):3018-3030. doi: 10.1002/hed.26339. Epub 2020 Jun 22. PMID: 32567746

We thank reviewer for the comment. The suggested references have been added in the discussion in the section ‘Current treatment regime’ line 17:



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“Although, recent reports highlighted that owing to heterogeneity in PD-L1 expression throughout tumours and utilization of different methods and antibodies, there might arise errors in immunohistochemical assessment of PD-L1 prior to therapy decisions^[89-91,92]. In course of extensively reviewing currently existing assessment methods across various cancers, Marletta *et al.* observed that in HNSCC, the registration trial utilized the 22C3 clone (Dako) on Agilent autostainer link 48 while the EMA granted administration of immunotherapy regardless of antibody and or the instrument used^[91]. The reports emphasized on establishment of a standardized uniform protocol considering the heterogeneity of expression as well as the antibodies and platforms used for assessment of PD-L1 before deciding whether immunotherapy should be administered to the patients^[89-91,92]”

As per instructions from the editor, we have also used a language editing tool Editage from the services suggested by the journal.

The manuscript is being submitted online with all the relevant files like Review article, figures, legends and table.

I hereby confirm that the article is not under consideration elsewhere.

I request you to consider our article for encouraging evaluation and publication in the journal. Furthermore, the review article will have a high impact on the readership of ‘World Journal of Stem Cells’ journal.

Thanking you,

With regards,

Sincerely,

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