Author's response to reviews

Title: Clinical significance of erythropoietin receptor expression in oral squamous cell carcinoma

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Author's response to reviews: see over
Dear Professor Chap, Senior Executive Editor:

Thanks for your correspondence on 18 March 2012. We are pleased to know that our manuscript entitled, “Clinical significance of erythropoietin receptor expression in oral squamous cell carcinoma” (Manuscript number: 7412438496356415), has generated such interest. We thank the reviewers for their helpful criticisms and comments, and have incorporated most of their suggestions into the enclosed revised manuscript. All the responses for the comments and changes of the manuscript have been listed in this file. The revised portions of the manuscript are highlighted in the revision note.

We look forward to hearing from you in the future. Please feel free to contact me if you have any questions.

Sincerely

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Reply to the reviewers’ comments

REVIEWER #1’s COMMENT
1. Because the Cox’s regression model revealed that only the T and N classifications were independent prognostic factors for survival, the authors have to make full discussion that advanced T and N could lead to tumor necrosis and hypoxia that resulted in high level of EPOR.

   High expression of EPOR is significantly correlated with advanced T classification and positive N classification. It represents the rapid proliferation of tumor cells may outpace the blood and nutrition supply. This probably leads to tumor necrosis and hypoxia in the microenvironment of tumor and may result into the higher level of EPOR.

2. Needs some language corrections before being published

   We had checked it by professional consultant in English.

REVIEWER #2’s COMMENT

1. Authors need to clarify what kinds of salvage treatment patients received.

   The treatment of oral cancer will be evitable to encounter the problems of local, regional or even distant failure. According to the guideline of our institute, the surgical salvage is the first choice if the tumor is operable. Otherwise, the patients will undergo salvage chemotherapy or chemoradiation if the tumor becomes unresectable although the prognosis of this group is dismal. We add this sentence to the Material and Method section.

   Authors need to correlate the EPOR expression with the disease free survival after surgery that would make the treatment impact not confused the study result.

   To avoid the impact of other adjuvant treatment in the survival, patients with high expression of EPOR still showed the poorer 5-year disease-specific survival significantly among patients underwent surgery only \( p=0.0079 \). We add this sentence to the discussion section.

2. Authors used four pair of tumor tissues and adjuvant non-tumor oral tissue to test the EPOR mRNA expression and the EPOR protein expression that shows the expression level is high in tumor tissue compared with adjuvant non-tumor oral tissue. However, the four pairs of samples are related small groups compared with the 256 OSCC patients they test in this study. Authors need to test more pair tissue samples to verify their important finding that advanced OSCC patients (T3, T4 or node positive) EPOR over expression rate is higher than early stage OSCC patients (T1, T2 node negative) and show the significance.
To clarify the point, we add more pairs of tissue for mRNA and protein expression of EPOR in the figure 2 and result section.

REVIEWER #3’s COMMENT

1. Abstract section:
   The authors need to describe the methodology (RT PCR, IHC, Western blot etc) they used to evaluate the EPOR expression in OSCC in detail.
   ⇒ We had added the following sentence: The mRNA and protein expression levels of EPOR in OSCC specimens were evaluated by Q-RT-PCR, Western blotting and immunohistochemistry assays.

2. Materials/ methods section:
   The authors need to provide more information on how they got the specimen for RT PCR and IHC analysis especially from which part of the tissues in advanced OSCC.
   ⇒ We had added the following sentence: To measure the expression of the EPOR in OSCC tissue and adjacent non-tumor tissues, fresh tissues were obtained from patients with OSCC biopsy specimens. These materials were histologically confirmed by frozen sections before quantitative RT-PCR, IHC, and western blotting assays.

3. Figure 1 Legend:
   The EPOR was seen not only in epithelial cells but also in inflammatory cells. Does this impact the expression level of EPOR? Please explain.
   ⇒ We had asked the pathologist to re-check our IHC slide which had stained EPOR. He found that the protein expression levels of EPOR in tumor tissues are stronger than that in the inflammatory cells, in epithelial cells or in adjacent non-tumor tissues. Even if EPOR appears in the inflammatory cells or epithelial cells; however, it expressed very low and it was not influence the EPOR-IHC scoring. Of course, it is suggested that EPOR may play role in inflammatory cells; however, it is another issue to investigate in the future.

4. Methods” section - IHC
   The statement of strongly (+++), moderately (++ or weakly (+) positive seems redundant as the authors defined high level of EPOR expression to be staining > 10% of the tumor cells, but not the positivity. However, the authors should also consider using some semi-quantitative methods including staining activity and staining cells number to determine the EPOR level.
The redundant part of the IHC classification is removed in the text. To determine the EPOR level by semi-quantitative methods by staining activity and stained cells number is a constructive advice. However, the determination of EOPR level by the method proposed by Li et al in reference 18 is simple and practical clinically.

5. The authors should rewrite the conclusions in the abstract section as they were not fully supported by the results.

High EPOR expression in OSCC is associated with an aggressive tumor behavior and poorer prognosis in the univariate analysis among patients with OSCC. Thus, EPOR expression may serve as a treatment target for OSCC in the future. We change it in the abstract section.