Reviewer’s report

Title: Bax Expression Measured by AQUAnalysis is an Independent Prognostic Marker in Oral Squamous Cell Carcinoma

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Reviewer: Wantao Chen

Reviewer’s report:

Resistance to apoptosis plays important role in the treatment failure of cancer. There are many studies have focused on the relationship of the patient prognosis and the expression level of Bcl-2, Bax and Bcl-XL and they have found that their expression closely related to patients’ prognosis. However, their results are not always consistent with each other due to the limitations of IHC methods and the different sources of test samples. This paper tried to use quantitative fluorescence immunohistochemistry based AQUAnalysis techniques to eliminate observer bias in IHC methods and they found the expression of Bax could be used as an independent predictor for the prognosis of OSCC patients. Their findings further validate the function of Bax in OSCC, however, this paper suffered some weakness at its current form.

Major compulsory revisions:

1. Since the radiation therapy is one of major treatment of OSCC, which definitely will influence the patients’ prognosis, the author should disclose some detail information about the patients’ treatment method, such as how to decide if radiation therapy is included or not included in the treatment of patients in this study.

2. Authors observed a positive correlation between Bax and Ki-67 and then proposed that patients with Bax overexpression maybe benefit from radiotherapy. From the data provided by this paper, this proposal is not suitable. Also, usually high expression of Ki-67 usually is linked to increasing proliferation and malignant behavior of cancer cells, in other words, poor prognosis of patients. The authors should give some explanations on this point.

3. In Tab1, since the sample number is not consistent in each group, the authors should include an explanation why the number is not consistent.

4. In Fig 1, since the size of tumor nests is obviously different between the middle and the lower row, the authors should explain how they justified the variation of the fluorescent intensity between them is not influence by the different size of the tumor nest?

Minor revisions:

In this paper, the authors used the median AQUA score in normal OSCE to define if the protein is overexpressed in tumor, but there are only 5 samples in normal OSCE group, the size is too small which may bring some bias.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests