Author's response to reviews

Title: Adenosine A2b Receptor Promotes Progression of Human Oral Cancer

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Author's response to reviews: see over
Dear Dr. Solera,

We are now sending our revised manuscript entitled “Adenosine A2b Receptor Promotes Progression of Human Oral Cancer” (Manuscript ID: 1934369491429500) by Kasama et al for consideration for publication in BMC cancer. We would like to thank you and the referees who have reviewed our manuscript and made comments and suggestions to improve the content. The critiques made by the reviewers are indeed helpful and constructive for improvement in our manuscript. The responses to the reviewer’s comments and concerns and summarizing the changes in the manuscript are indicated below.

I hope that our current paper based on these responses and corrections are now satisfactory for consideration for publication in BMC cancer.

We look forward to a favorable response from you.

With my best regards,

Respectfully yours,

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Reviewer #1

We would like to thank the referee who has reviewed our manuscript favorably and we appreciate the comments and suggestions to improve the content. We have revised the manuscript as indicated below to address the points raised by the reviewer. The following are our specific responses.

Major compulsory revisions

‘In the methods section, the method chosen to assess cell growth is cell counting. This has obvious disadvantages as it does not separate the possible mechanisms of changes in cells number—this may be by increased proliferation or by reduction in cell death.’

Response:

As has been suggested by the reviewer, we assessed cell growth using the 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. We have added an explanation of MTS assay in the Methods section.

‘In order to complete the presented links between HIF1α and ADORA2b and number of other experiments would be very useful and would add to the paper: ’

1. Repeat the data presented in Figure 6 under hypoxic conditions. If the hypothesis presented is correct, the increase in ADORA2b and HIF1α under hypoxia should be abrogated/markedly reduced in the ADORA2a knockdown cells. Again, as the stably transfected cells are available, this could be easily done.

Response:

As pointed out, we observed the expression status of HIF-1α in the ADORA2B
knockdown cells under hypoxic conditions (Figure 6) and modified some part in the Results (page 20, line 13) and Discussion section (page 24, line 6).

‘2. Correlate the expression of ADORA2b in the tissues with HIF1a expression by IHC in the same samples.’
Response:
We agree with the reviewer’s suggestions. The correlations between ADORA2b and HIF-1α must be important for our scientific field. Therefore, we would like to consider them for our future work.

Minor essential revisions
‘P8 L15: are these cells spontaneously immortal or have they been artificially immortalised?’
Response:
These cell lines are a spontaneously immortal cell line derived from human oral cancer.

‘P15 L1: please state the level of agreement between the 2 pathologists’
Response:
The pathologic diagnosis each OSCC sample were done together by two pathologists. We adopted samples where both pathologists gave identical diagnoses.

‘Figure 1: some comment on the mismatch of RNA and protein expression should be made, especially for Ho1-N-1 and KOSC2 cells. Is there an explanation for
For the discrepancies in mRNA expression and protein levels in Figure 1A and 1B, we have added the following sentence “A significant up-regulation of ADORA2B was detected in all OSCC-derived cells examined at protein levels (Figure 1B), indicating that ADORA2B expression is necessary for oral carcinogenesis. On the other hand, the mRNA expression states depended on the cells (Figure 1A). A possible explanation for this discrepancy is that ADORA2B proteins are differently affected by the post-translational ubiquitination and proteolysis in each tumor cell lines. In this context, discrepancies between mRNA expression levels and protein levels has been reported in OSCC cellular lines (reference 33).” in the Discussion section (page 22, line 15).

‘Figure 2: in the version I had for review, the immunohistochemistry figures are of poor quality. These should be improved and a high and low power for each presented to allow assessment of localisation of expression.’
Response:
According to the reviewer’s suggestion, we have changed Figure 2A, B.

‘Table 1. If clinical outcome data is available, this would also add to the paper. Is it would lend weight to the possible usefulness of ADORA2b in OSCC’
Response:
As requested, we added the survival analysis data in Figure 2D.
Reviewer #2

We would like to thank the referee who has reviewed our manuscript and we appreciate the comments and suggestions to improve the content.

We have revised the manuscript as indicated below to address the points raised by the reviewer. The following are our specific responses to the reviewer.

‘Page 6, line 16. This sentence doesn’t seem to be correct: Adenosine A2b receptor (ADORA2B), classified into four subfamilies (ADORA1, 2A, 2B, 3).’
Response:
As has been suggested by the reviewer, we have changed the original sentence ‘Adenosine A2b receptor (ADORA2B), classified into four subfamilies (ADORA1, 2A, 2B, 3)’ to the corrected sentence ‘The adenosine receptors consists of four members that belong to the G protein-coupled receptor superfamily, including A1, A2A, A2B and A3’ (page 7, line 2).

‘Figure 1. How are the HNOKs cultures established? Are they primary cultures or established “normal” keratinocyte cell lines? Are they derived from normal tissue of cancer patients or from healthy donors? I would expect to see at least two independent samples of HNOKs for reproducibility of the data.’
Response:
As has been pointed out, we have added a detailed explanation of HNOKS cultivation in
the Methods section (page 9, line 15). We used three samples independent samples of HNOKs for reproducibility of the data.

‘Figure 5. Is HIF-1α induced by hypoxia in OSCC cells?’

Response:

As has been suggested by the reviewer, we have assessed the expression of HIF-1α under hypoxic conditions in OSCC cellular lines and modified some part in the Results (page 20, line 13) and Discussion section (page 24, line 6).

‘The authors show nicely that hypoxia induces ADORA2B. Did they see an increase in proliferation as well?’

Response:

Your suggestions are very valuable, and we would like to consider them for our future work.

‘Figure 6. Total Erk and Akt seem to be downregulated in HSC-3 cells after ADORA2B suppression. The westerns should be quantified and total Erk and Akt normalized with the loading control.’

Response:

As requested, we have quantified these protein expression and normalized with the loading control (Figure 6).

‘Figure 6. Is HIF-1α transcription regulated by ADORA2B?’
Response:

This phenomenon has been reported by several theses. HIF-1a is a common transcription factor induced by ADORA2B activation in inflammatory bowel disease and urologic diseases (reference 37, 38).

‘Figure 6. Is the induction of HIF-1a by hypoxia prevented in ADORA2B-suppressed cells?’

Response:

As has been suggested by the reviewer, we assessed the induction of HIF-1a by hypoxia prevented in ADORA2B-suppressed cells (Figure 6).