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

‘1. The issue of cell numbers and proliferation. This is not satisfactorily addressed by MTS assay, which uses mitochondrial activity as a surrogate for cell number. It still does not answer the outstanding question – does proliferation or apoptosis change? These will have to be measured directly.’

Response:

As has been suggested by the reviewer, we assessed apoptosis using the Annexin V-FITC Apoptosis Detection kit (Abcam, Cambridge, MA, USA). We have revised the Methods and Results sections (page 14, line 4; page 20, line 9).

‘2. It is good that the survival data has been added, but if ADORA2B expression is so closely linked to HIF1α expression then surely the data will need to be expressed in terms of high and low HIF1α? Thus, the data on HIF1α expression is needed to fully understand this data.’

Response:

As the reviewer requested, we have assessed the expression level of HIF-1α protein in OSCC clinical samples. We certified that HIF-1α was high expressed in 19 out of 20 ADORA2B-positive OSCC samples. We believe the result helps our in vitro data
which the expression of ADORA2B is so closely linked to the expression of HIF-1α expression in OSCCs. We have added the data in the Methods section, Results section, Discussion section, and Additional files 1 (page 15, lines 2; page 22, line 4; page 24, line 16).
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.

‘HNOKs cultures: the authors mention that HNOKs were obtained from 3 patients. It is unclear whether RNA and protein experiments in Fig1 were done with HNOKs from different patients. Was RNA or protein pooled from different patients?’
Response:
RNA and protein were pooled from same patients. We have added comment in the Methods section (page 11, line 5).

‘Page 10, line 4: “The specimens were healthy oral mucosal epithelium”. This sentence probably applies to the previous paragraph and should be corrected.’
Response:
As suggested, we have deleted the repeating sentence and modified part in the Methods section (page 10, line 4).