Author's response to reviews

Title: STK33 overexpression in hypopharyngeal squamous cell carcinoma: Possible role in tumorigenesis

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Dear Prof. Annie Lyn Bravo:

Thank you so much for your kind e-mail, indicating our manuscript (MS: 1100756891412223), entitled “STK33 overexpression in hypopharyngeal squamous cell carcinoma: Possible role in tumorigenesis”, will be considered for publication after it is properly revised according to the reviewers’ comments and suggestions.

Below are our point-by-point responses to the reviewers’ comments.

To editor:

We really appreciate the editor’s objective comments on our manuscript.

STK33 gene, which is discovered by comparative genome sequencing of the human chromosome 11 region 11p15 and encodes a serine/threonine kinase, is a new and important gene relevant to tumors. Its precise role in tumorigenesis, however, has been still controversial to date. The present study was performed on both a hypopharyngeal squamous cell carcinoma (HSCC) cell line (Fadu cells) and the resected specimens from patients with HSCC, with an attempt to explore the possible role of STK33 gene in HSCC. This report represents a pilot study on this subject concerning the traits of STK33 gene in HSCC and further research on this issue is being systematically carried out in our Lab.

According to the reviewers’ thoughtful comments and suggestions, we have tried our best to revise our manuscript and sincerely hope that the newly presented version will reach your threshold for publication in the journal.

To reviewer #1:

We appreciate the reviewer's suggestion.

According to the reviewer’s suggestion, in the newly revised text, we have tried our best to avoid the grammatical and typographical errors and, additionally, we
have invited a native speaker, who is teaching English at Shandong University, to polish the whole manuscript.

To reviewer #2

We appreciate the reviewer's comments and suggestions.

1) This is an excellent suggestion. Currently, an in vivo study, one of several essential efforts on this project, is ongoing in our lab, with already achievement of certain preliminary results exhibiting a tendency in accordance with the data from in vitro experiments.

2) In the original text, the description of the figure 4 did not presented the time point at which STK33-RNAi significantly increased the expression of Caspase. In the newly revised text, according to the reviewer's suggestion, the time point, i.e., 96 h post-transfection, has been added in the legend of figure 4 (Legends, Page 39, Line 830). This has been also described in the section of Results (Results, Page 19, Line 417).

3) Histopathologically, tumors are graded into keratinizing SCC, which is characterized by large tumor island with variable “pearl” formation pushing margins and, non-keratinizing SCC, which is characterized by scattered small irregular cords or single tumor cells, with poorly defined infiltrating margins (please see this on page 17, from Line 354 to 358, Reference 17). Keratinizing HSCC is well differentiated and associated with a better prognosis, whereas, non-keratinizing HSCC is poorly differentiated and associated with a more aggressive course. In the present work, the expression of STK33 was markedly elevated in non-keratinizing type than that in keratinizing type. Therefore, the significance of the greater expression of STK33 in non-keratinizing HSCC than in keratinizing type is that STK33 might be related to the dedifferentiation in HSCC (please see this in the section of discussion, Page 22, Line 474-479).

4) Seeing the reviewer’s comment on figure 6 B, we meticulously rechecked and recalculated the raw data. Statistical analyses showed that there was a significant reduction in STK33 expression when cells were subjected to STK33-RNAi.

To reviewer #3:

We really appreciate the reviewer's constructive comments and suggestions.

1) This work comprised 30 cases of HSCC, which was a small sample size. In deed, the credibility might be affected with small samples. To maximally overcome the limitation in the case of given small sample size, a new observation method, which counts each lesion with a fraction containing area of carcinoma under microscopic vision fields in each case, was employed for pathological statistical analysis (Materials and methods, Page 9, Line 190, Reference 14). In the current study, we adopted this modality to determine the STK33 expressions in all separated fields of each slice with IHC staining under light microscope at a magnification of x 400. Thus, though our material consisted of 30 randomly chosen specimens, the fields' numbers of keratinizing and non-keratinizing types reached up to 888 and 552, respectively. We had explained the number of fields at the end of table 1 (please see “c”).
Keratinizing and non-keratinizing types are graded at histopathological levels. According to criteria issued by American Joint Committee on Cancer (AJCC, 2010), the clinical stages of HSCC are synthetically judged by the size and the invasive deepness of tumor, the location and the size of the metastatic lymph node, the distant metastasis or not, and etc. As the clinical stage is distinct from histopathological grades in the criteria by AJCC, we estimated the STK33 expression in histopathological grades and clinical stages, separately.

2) In general, the judgement of IHC staining includes two aspects, i.e., the proportion of positive epithelial cells and the staining intensity and, importantly, only consideration of either positive cells or positive strength is likely to result in the inaccurate results. In this regard, we utilized the “H-score approach” to estimate the STK33 expression as described in details in the section of “Materials and Methods” (Page 9, Line 184-191). Meanwhile, as a final score, which was calculated by multiplying category A by B, was able to estimate the STK33 expression more comprehensively and accurately, thus, we took the final score, rather than category A or category B, separately, as raw data for statistical analysis and, finally, determined the STK33 expression in HSCC as well the correlation of STK33 expression with clinicopathological characteristics in this work.

3) STK33 gene, as a relatively new gene, has been received considerable attention in tumor research area, but, its exact roles of this gene are still debatable in tumorigenesis. In the present study, we mainly focus on determining the properties of STK33 gene in HSCC using a cell line (Fadu cells) and human specimens as experimental materials. The preliminary results from this work support that STK3 is a potential oncogene, thereby influencing the tumor biological behaviors, such as apoptosis, proliferation, and etc. As the cell lines of the HSCC are of rather rareness, in this work, we only used Fadu cells to investigate the basic functions of STK33 in HSCC. If the present study had further extended to other types of cancer cell lines, this article would have, no doubt, come in for a great deal of significance. We will surely consider your suggestion in our future study.

4) Pathologically, keratinizing HSCC is well differentiated and associated with a better prognosis, whereas, non-keratinizing HSCC is poorly differentiated and associated with a more aggressive course. In this work, we showed that the expression of STK33 was markedly increased in non-keratinizing type than that in keratinizing type. Therefore, the significance of the greater expression of STK33 in non-keratinizing HSCC than in keratinizing type is that STK33 might impact the dedifferentiation in HSCC (please see this in the section of discussion, page 22, line 474-479).

Lastly, with respect to the resected specimens, 30 cases (precluding chemotherapy or radiotherapy) were randomly selected from nearly 100 patients, who underwent operation by our team. To tell the truth, such samples have been possessed by each expert who performed the operation. So, it is really difficult to obtain more samples in a short period.

In addition, we have revised the article according to your journal’s style.
All major changes have been marked with red color in the newly revised text. We hope all the changes will be satisfactory and our manuscript will be finally published in your esteemed journal.

Thank you so much for your kind consideration.

Best wishes,

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