Author’s response to reviews

**Title:** SET domain containing protein 5 (SETD5) enhances tumor cell invasion and is associated with a poor prognosis in non-small cell lung cancer patients

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Editor Comments:

1. Please change the Materials and Methods heading to Methods.

Answer : Thank my dear editor for your comment, we change the Materials and Methods heading to Methods.
2. Please clarify in the Ethics approve and consent to participate section whether the consent was written or verbal. If verbal, please state the reason and whether the ethics committee approved this procedure.

Answer: Consent was written in the Ethics approve and consent to participate section was added to the original manuscript.

3. The Availability of data and materials section refers to the raw data used in your study and presenting tables and figures is not sufficient to state that all data is contained within the manuscript and additional files. Please only use this statement if you have indeed provided all raw data on which your study is based. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

• The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

• The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

• All data generated or analysed during this study are included in this published article [and its supplementary information files].

• The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

• The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Please note that if you do wish to share your raw data and do not have consent from all patients to publish this data it will need to be de-identified.

Answer: Thank my dear editor for the rigorous work attitude. The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.
Please also note that if you include your raw data as a supplementary file you will need to provide, after the References, a section titled “Additional files” where you list the following information about each of your supplementary files: * File name (e.g. Additional file 1), * Title of data, * Description of data. All additional files will also need to have been cited in the main manuscript.

BMC Cancer operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Xiu-Peng Zhang (Reviewer 1):

The manuscript titled with "SET domain containing protein 5 (SETD5) enhances tumor cell invasion and is associated with the poor prognosis of non-small cell lung cancer " is well designed and performed properly, they indicate that SETD5 may enhance lung cancer invasion and metastasis by promoting ERK and its downstream P90rsk, they have both in vitro data as well as human data which all indicated SETD5 may play an important role in lung cancer.

However,

1. I think if they can improve the English write, the reader would be benefit a lot.

Answer : Thank the reviewer for his/her suggestions. Because our mother tongue is non-English, the manuscript had been polished by the professional institution before submitting.

2. I wonder if Figure 2g should be the same style with the Figure 1, then author should list the number of the patients in different survival time as the figure 1.

Answer : We had listed the number of the patients in different survival time in Figure 2g.

3. As we know, the adenocarcinoma and squamous cell carcinoma are originating from different kind of cell types and if the authors can show whether the relationship between STED5 expression and histology of lung cancer, it would be helpful for the reader to understand more.
Answer: In order to make the reader understand the article more clearly, detailed data analysis has been carried out in Table 1. Thank the reviewer for his/her thoughtful guidance.

Jianfei Huang (Reviewer 2):

1. How large is the area in the figure 3c that cell number you provided? Please indicate on the ordinate or in the article.

Answer: Thank the reviewer for his/her guidance. We have randomly selected views under mirror and the average were calculated. Markers are placed in the lower right corner of each graph in the figure 3c.

2. What is the numerical representation of the ordinate in Figure 3b?

Answer: it had been labeled in Figure 3b, it is the migration distance.

3. Figure 3b is missing the ruler, please add.

Answer: Thank the reviewer for his/her thoughtful guidance. The ruler had been added.

Reviewer 2 (Reviewer 3): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

No - there are minor issues

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

No - there are minor issues
EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

No - there are major issues

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?

Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: In the presented study the authors investigated the role of SETD5 on NSCLC aggressiveness and survival time followed by experimental, clinical and literature sets. This study is interesting because NSCLC is still a leading issue for in contemporary oncology. However, I have some concerns with the methods of this study.

Answer: Thank the reviewers for reading our articles carefully and making valuable suggestions.

REQUESTED REVISIONS:

1. The major point is analysis and interpretation on enrolled patients. First of all 147 patients underwent surgery, but only 48 Non-cancerous tissues were obtained. In the table representing the studied group, we see the TNM staging. It is not according to 7th edition how authors suggested in material and methods section. The question is specific to the group marked as "III". Is it a IIIA group? Such patients only under the special condition could be qualified to surgery. Moreover, according to recent guidelines, IIIA patients should undergo neo- or adjuvant therapy.
Therefore, please compare only patients I vs II (III may be independent unfavorable factor of OS). Additionally, some information about patients' history after surgery should be added - "all patients received CTH after surgery..." was it adjuvant or maybe they were scheduled to CTH in the future when disease progressed? Any regimens of CTH should be added.

Answer: Tissue samples were obtained from 147 patients who underwent complete surgical excision at the Cancer Hospital of China Medical University from 2009 to 2011. All specimens were diagnosed as lung squamous cell carcinoma or lung adenocarcinoma. No patients had received neoadjuvant radiotherapy or chemotherapy, and all patients received chemotherapy after surgery. The adjuvant chemotherapy was started from 3 to 4 weeks after operation. Chemotherapy regimen: NP, GP regimen or drug sensitive gene test results. In principle, platinum-containing two-drug regimen should be applied. Chemotherapy cycle: generally 4-6 cycles. We think patients I+ II vs III could express that overexpression of SETD5 is associated with advanced TNM stage, lymph node metastasis, and poor prognosis of patients with NSCLC more clearly.

2. Please clarify the division of patients to positive/negative of SETD5. "A final score of 0-12 was obtained by multiplying the intensity and percentage scores" - if you multiplied the total should be 14? and in next sentence "Tumors were seen as positive for SETD5 expression with a score ≥4", so if 4 per 12 were passed patient was considered as positive? For me it is a little confusing. I also suggest for survival analysis: three groups according to points: negative(weak), moderate, positive (strong).

Answer: Thank my dear editor for the good suggestion. Staining intensity was scored as 0 (no signal), 1 (weak), 2 (moderate), or 3 (high). The percentage of cells stained was scored as 1 (1-25%), 2 (26-50%), 3 (51-75%), or 4 (76-100%). A final score of 0-12 was obtained by multiplying the intensity and percentage scores. The negative and positive groups can express the function of SETD5 clearly.

Minor points:

2. Large cell carcinoma patient should be excluded from the study, its presence does not affect results.

Answer: Large cell lung cancer belongs to non-small cell lung cancer in Pathologically. We believe that it should be retained?
2. In some samples the differences of SETD5 was found - cytoplasm rather than nucleus - please discuss why?

Answer: This study showed that SETD5 was strongly expressed in both cytoplasm and nuclei of NSCLC specimens, while SETD5 expression in normal lung tissues was lower. The expression of SETD5 was associated with clinicopathological factors and poor OS. Taken together, these results indicated that SETD5 may be an oncogenic factor, as supported by the oncogenic role of other proteins of the SET domain protein family. This is one of the specific mechanisms that we will further study later.

3. Limitations of the study should be added, and potential effect of SETD5 inhibition in cancer therapy.

Answer: Limited to the number and time of patients included, this experiment may have some limitations. However, the study of SETD5 will not end. We will continue to explore the molecular biological functions of SETD5. In the future, SETD5 may be a new tumor marker, and may also play a role in explaining the mechanism of drug resistance in patients undergoing chemotherapy.

4. There are language mistakes, that need to be polished before publication.

Answer: We have polished our contributions in English before submitting them.

Note: This reviewer report can be downloaded - see attached pdf file.