Author's response to reviews

Title: Analysis of B7-H4 expression in metastatic pleural adenocarcinoma and therapeutic potential of its antagonists

Authors:

CHENG CHEN (210332029@suda.edu.cn)
QIUXIA QU (quqiuxia81@163.com)
FANG XIE (xiefang2@suda.edu.cn)
WEIDONG ZHU (zhuwdsz@163.com)
JIANAN HUANG (huangjian_an@163.com)
YEHAN ZHU (zhuyhans@sina.com)

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Author’s response to reviews:

Dear Editor

We are very grateful to the editor and reviewer for their constructive comments on our manuscript entitled “Analysis of B7-H4 expression in metastatic pleural adenocarcinoma and therapeutic potential of its antagonists”. We appreciate the opportunity for us to provide further clarification for publication in BMC CANCER. We have carefully addressed the questions raised in the comments, point by point.

I wish the paper can be published in your professional magazine and I expect your reply.

Best wishes to you!

Sincerely,

Cheng Chen, MD
Vice-director of Respiratory Department, the First Affiliated Hospital of Soochow University

Response to Editor
1 - Please rename the "Introduction" section "Background," and "Materials and methods" "Methods"

Answer: Background section line 1, page 3, Methods section, line 35, page 3

2 - Please include a "Conclusions" section

Methods section, line 25-33, page 8

3 - Please include a list of abbreviations used in the manuscript before the Declarations section

Answer: Line 1-8, page 9

4 - For all research involving human subjects, informed consent to participate in the study should be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript.

Answer: Declarations section, line 10-16, page 9

5 - Please include an Availability of Data and Materials section in the Declarations;

Answer: Declarations section, line 19-22, page 9

6 - Please use initials rather than full names in the Author Contributions section;

Answer: Declarations section, line 1-5, page 10

7 - Please include a statement in the Authors' contributions section to the effect that all authors have read and approved the manuscript, and ensure that this is the case.

Answer: Declarations section, line 1-5, page 10

8 - Please check our authorship criteria and ensure all named authors have provided sufficient input to be so registered.

Answer: checked.
9 - Please indicate the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript;

Answer: Methods section, line 25-32, page 9

10 - Please re-write the following sections, which overlap with text in other, previously published papers: Introduction: “Pleural effusion (PE) is a common clinical condition caused by malignant tumors, as well as some non-malignant diseases [1].” Results: “A total of 32 eligible patients were enrolled. Baseline characteristics of the subjects were listed in Table 1.”

Answer: Background section, line 2, page 3, Results section, line 3, page 6.

11 - In figure 2, the B7-H4 panel does not appear to us to contain the stained cell in the upper left hand corner corresponding to the cell with the yellow arrow in the Merge panel; please clarify

Answer: In figure 2, upper left hand corner corresponding to the cell was the panel of nuclear staining (DAPI, blue), upper right hand corner corresponding to the cell was the panel of B7-H4 staining (red), lower left hand corner corresponding to the cell was the panel of merge (yellow).

12 - At this stage, please upload your manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethrough or text in different colours. All relevant tables and figures should also be clean versions. Figures (and additional files) should remain uploaded as separate files. Should you wish to respond to these revision requests, please include the information in the designated input box only.

Answer: Done.

13 - Please ensure that the order of authors on the manuscript and that in the submission system match

Answer: we made sure the order of authors on the manuscript.

Response to Reviewer

Reviewer 1: The authors have explored the expression of an important regulatory molecule, B7-H4, in malignant pleural effusion (MPE), and started to elucidate its functions in this particular type of malignancy. This is a potentially important study.

There are a few (major and minor) issues in this study:
1 - Major issue is the data presentations. (1). Figure 4C. No P value is presented. (2). Figure 5. A better way to present the data is to show data (CT scan) in both groups of mice, before and after treatment with either isotype control mAB or anti-B7-H4 mAb.

Answer: (1) We presented the P value in Figure 4C. (2) We mimicked quantitative assessment of MPE as clinic by using CT scan. At the same slice, we can assess and semi-quantitative the MPE by fluid sonolucent area noticed in mediastinal window. CT images before and after treatment with either isotype control mAb or anti-B7-H4 mAb were presented, see line 5-9 page 7, 8-23 page 8.

2 - Better Introduction and Discussion, with a few more references:


B. Most studies have shown an inhibitory role of B7-H4 in the antitumor immunity, while a few studies have pointed to the opposite. In this regard, it is good to discuss this issue and cited a few relevant papers. (1). Rahbar R, … Ohashi PS. B7-H4 expression by nonhematopoietic cells in the tumor microenvironment promotes antitumor immunity. Cancer Immunol Res. 2015; 3:184-95. (2). Kreymborg K, et al. Ablation of B7-H3 but Not B7-H4 Results in Highly Increased Tumor Burden in a Murine Model of Spontaneous Prostate Cancer. Cancer Immunol Res. 2015; 3:849-54.

Answer: More references were cited in the text, to deepen the background section and discuss section (page 10-12, ref 8, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23). Better Introduction and Discussion was present in revised version ( line 15-16 page 3, line 23-29 page 3, line 15-19 page 5, line 13-24 page 7, line 8-23 page 8).

Reviewer 2: The study’s data was in support of the significance of B7-H4 expression in MPA, for clinical reference significance; However, the experimental design and data presentation suffer from several major weaknesses:

1 - The small sample size

In this study, only 23 patients with metastatic pleural adenocarcinoma (MPA) and 9 patients with early-stage lung adenocarcinoma were included, which is very unlikely to have the adequate statistical power to draw any conclusive statement that may actually reflect the status in the general population.

Answer: As stated in Discussion section, “this may be possible when B7-H4 was investigated on a larger number of cases in the future”. However, we get some statistical data based on the data
available from the small sample size, including correlation of B7-H4 and Ki-67 in MPA, and difference of B7-H4 expression between MPA and early stage lung cancer. Median survival for high nuclei membranous B7-H4 patients was 10 months and 15 months for B7-H4 low patients, indicating that nuclei membranous B7-H4 expression has possible impacts on survival of MPA patients. Due to low patient number here, it would attach statistical significance if we expanded the sample size (line 30-34 page 6, line 1-3 page 7).

2 - Table1: MPA related to clinical pathological parameters is too little, and not to make a correlation analysis, is just an appearance, no practical significance.

Answer: We presented the statistical data about the clinical pathological parameters in the revised version (Table 1).

3 - fig.1 Image resolution is not enough, since the article in the MPA B7-H4 and Ki67 correlation, should be added with the same location Ki67 immunohistochemical images to do contrast.

Answer: We presented the Ki-67 staining in the revised version, which better the location of B7-H4 (Fig 1 and legend 1).

4 - Fig.4C corresponding to the total survival time of patients with MPA median or mean and the clinical pathology-related factors and B7-H4 expression of Univariate analysis should have a table representation, so that more credibility.

Answer: We presented the P value of B7-H4 expression in OS analysis. B7-H4 expression of x2 analysis was shown in Table 1.

5 - There are a number of spelling and grammatical errors along with inadequate presentations.

Answer: We invited Prof. Jim Xiang (Cancer Research Unit, Saskatchewan Cancer Agency, University of Saskatchewan, Saskatoon, Canada) to deal with spelling and grammatical errors.

Once again, we would like to thank you for your concerns and the critical reading of our paper, and we would continue to keep close attention to your journal with interest.

Sincerely,

Dr. Cheng Chen, MD

Vice-director of Respiratory Department, the First Affiliated Hospital of Soochow University