Author’s response to reviews

Title: CTHRC1 Induces Non-small Cell Lung Cancer (NSCLC) Invasion through Upregulating MMP-7/MMP-9

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Author’s response to reviews:

Responses to Barbara Mary Fingleton (Reviewer 1):

Comment 1: This revised manuscript from He et al addresses some but not all the previous reviewer comments. Most responses instead suggest that questions raised will be answered in follow-up studies. While additional experimentation cannot always be done, the authors should at the very least address some of the points raised in the discussion. For example, they should discuss the unusual apparent nuclear localization of CTHCR1, and also the possibility of the driver mutation (e.g. EGFR or k-Ras) in the cell lines or cancers being a critical regulator of the downstream effect of CTHCR1.

Response 1:

1). According to your request, we spent almost two weeks to add the additional data showing the nuclear localization of CTHRC1 by western blot (showed in Supplementary Figure S5, Line 10 and L231-233). Furthermore, we provided corresponding opinions in discussion section (highlighted in Line 501-504).

2). About the possibility of the driver mutation (e.g. EGFR or k-Ras) in the cell lines or cancers being a critical regulator of the downstream effect of CTHCR1, our team will do our best to elaborate it in the future because that is a tremendous research work.

Comment 2: Additionally, the authors have taken the unusual step of removing a significant part of the data provided in the initial submission, i.e. the mouse studies. However there are still some sections that retain description or discussion of mouse studies that need to be removed. These include lines 333-347 in the results as well as the corresponding figure sections 3I and 3J. Also in the discussion, lines 565/566 and 575-577 suggests that CTHCR1 causes metastasis, but that has not been established in this manuscript. In this regard, the title of the manuscript should also be changed to remove 'and metastasis'.
Response 2:

1). We have removed or corrected the description in result section or discussion of mouse studies.

L309-313 in Results Section.

L521-522 and L529-531 in Discussion Section.

2). We also deleted the corresponding Figure sections 3I and 3J and their legend (highlighted in L819-821).

3). According reviewer’s suggestions, the title of the manuscript was changed to “CTHRC1 Induces Non-small Cell Lung Cancer (NSCLC) Invasion through Upregulating MMP-7/MMP-9” (highlighted in Line 1-2)

Comment 3: Finally, reviewer 3 refers to 'MMP4', which is included as a row in figure 3A,B. There is no matrix metalloproteinase-4 (this name was originally assigned to an already named member of the family and so has been deleted). A protein that has 'MMP4' amongst its many alternative names is interleukin enhancer binding factor-3 (ilf3), but this is completely unrelated to the proteinases implicated in tumor invasion. Therefore the inclusion of "MMP4" in figure 3A,B is misleading and should be removed.

Response 3: Sorry, this is a spelling mistake (mistake MMP3 as MMP4) and we have corrected in Figure 3A and B.
Responses to Takashi Kijima (Reviewer 2):

Major Comments

Comment 1: The revised manuscript and the authors' response letter have addressed some of the previous issues that were raised. However, no updated data by additional experiments that I requested has not appeared in this revised form yet. I request the authors to perform additional experiments and faithfully answer the questions one by one. Especially, I strongly recommend the authors to add the data showing the nuclear localization of CTHRC1 by western blot or immunofluorescence staining before publication. CTHRC1 is generally recognized as a secreted protein. Therefore, the scientific impact and lucidity of this paper will be much higher if the authors can clearly show the nuclear localization of CTHRC1. I know that ChIP assay indirectly reflects the nuclear localization of CTHRC1 as the authors commented in their response letter. I still think that it is necessary to show the direct evidence of the nuclear translocation of CTHRC1.

Response 1: Thank you so much for your good suggestions. According to your request, we spent almost two weeks to add the additional data showing the nuclear localization of CTHRC1 by western blot. (showed in Supplementary Figure S5, Line 10 and L231-233)

Responses to Kęstutis Sužiedėlis (Reviewer 3):

Comment 1: Despite that authors did not respond to my comments point by point, they took care about most of my comments/queries and revised the manuscript adequately. In the revised manuscript, however, authors state that significant difference in an adhesion was observed between CTHRC1-overexpressing cells and pcDNA3.1-vector-transfected cells when both 7 and 9 MMPs were downregulated. This statement is not supported by the data provided in Fig. 4A. Observation of the same experiment that significant difference is monitored in Transwell and scratch assays between CTHRC1-overexpressing cells and pcDNA3.1-vector-transfected cells when both 7 and 9 MMPs were downregulated suggests targets of CTHRC1 other than MMPs 7 and 9 important for cell migration or alternatively technical problem of an experimental design. Perhaps it would help if authors could comment on this observation.

Response 1: This is an excellent comment, which will significantly improve the quality of manuscript. After detailed comparison Figure 4 and corresponding content in the article, we completely revised the primary content highlighted in Line 326-337 as following:
“However, when MMP7 or MMP9 was knocked down in CTHRC1-overexpressing cells, respectively, they exhibited significant difference in invasion ability compared to those CTHRC1-overexpressing cells without MMP7 or MMP9 knock-down (Figure 4B, 4C). Additionally, significant difference was observed in a scratch assay between CTHRC1-overexpressing cells with and without MMP7 or MMP9 knocked-down, respectively (Figure 4D-4F). More significant difference was observed in a adhesion, Transwell and scratch assay in CTHRC1-overexpressing cells with both MMP7 and MMP9 knocked-down, compared with those with MMP7 or MMP9 knocked-down, respectively. Furthermore, we did not observe the changed expression of MMP7 or MMP9 when MMP9 or MMP7 was knocked down (Supplementary Figure 5C, 5D).”