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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Responses to Editor Comments:

Comment 1: Please add all authors’ email addresses before the abstract.
Response 1: We have added all authors’ email addresses (Line 32-41).

Comment 2: On uploading your revisions, please remove all tracking/highlighting and instead upload a single clean version of the manuscript.
Response 2: We will upload a single clean version according to your request.

Comment 3: Under 'Ethical approval and consent to participate' (within declarations) please add your ethical approval reference number.
Response 3: We have added the ethical approval reference number (Line 555-556).

Comment 4: Under Funding (within declarations) - please add the phrase: 'The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript'.
Response 4: We have added the phrase in Line 582-584.

Responses to Barbara Mary Fingleton (Reviewer 1):

Comment 1: The authors have made many of the changes requested, including the title change and the removal of references to mouse experiments. However there are still some inappropriate references to the work supporting a role in metastasis within the abstract, and near the end of the introduction (e.g. line 129).
Response 1: We have corrected some inappropriate references to the work (Line 64-68 in Abstract and Line 129 in Introduction).

Comment 2: More critically, however, the experiment requested by reviewer 2 (establishing nuclear localization of CTHRC1) as purportedly shown in Fig S5, does not have the appropriate controls to establish nuclear localization. At the very least lanes to show the total and cytosolic
fractions need to be shown to establish that there is indeed a enrichment within the nucleus. Response 2: Histone 2 was used the inner control of nuclear protein and α-tubulin was used the inner control of cytoplasmic protein in Supplementary Figure S5 (Supplementary Matirials Line 229-234).

Responses to Takashi Kijima (Reviewer 2):

Comment 1: The manuscript has been revised well. I think this manuscript will be acceptable after the following two corrections have been made. First, regarding the western blot of Supplementary Figure S5 showing nuclear localization of CTHRC1, the authors need to demonstrate that cytoplasmic protein has been removed from their nuclear protein samples because, according to their previous paper (Ke Z et al. Oncotarget 2014, 5, 9410-9424.), CTHRC1 is thought to be abundantly located in cytoplasm. I recommend performing western blot using internal controls of not only nuclear protein but also cytoplasmic protein. Alpha-tubulin used in other experiments can be a reliable internal control of cytoplasmic protein. Additionally, the whole cell lysate samples also need to be included as input. Otherwise, the authors cannot rule out the possibility of the contamination of cytoplasmic protein in their nuclear protein samples. Second, the authors should mention how they isolated and extracted nuclear protein from the cells in "Methods and materials" or "Supplementary methods and materials" section so as to be reproducible..

Response 1: 1). Histone 2 was used the inner control of nuclear protein and α-tubulin was used the inner control of cytoplasmic protein in Supplementary Figure S5 (Supplementary Matirials Line 229-234).

2). Cell nucleoprotein was extracted using EpiQuik Nuclear Extraction Kit (Epigentek, Farmingdale, NY) according to the manufacturer’s instructions (Line 172-174).