Reviewer’s report

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

**Version:** 0  **Date:** 20 Jun 2017

**Reviewer:** Takashi Kijima

**Reviewer's report:**

To the authors

In the present study, the authors revealed that CTHRC1 increased the ability of invasion and migration of lung cancer cells and promoted metastasis through MMP7 and MMP9 upregulation. They also demonstrated the significant correlation between CTHRC1 expression and poor prognosis in patients with NSCLC.

They had already shown in their previous paper that CTHRC1 enhanced anchorage-independent growth of lung cancer cells and could be a negative prognostic factor in NSCLC (Ke Z et al. Oncotarget 2014, 5: 9410-9424.). Therefore, the conclusion of this study is not so novel. However, it is worth discussing about publication in BMC cancer because they revealed another mechanism of CTHRC1 which led to a poor prognosis in NSCLC.

Although their experimental data are generally convincing and the sample size of the patients they analyzed is enough to acquire their conclusion, they need to clarify some issues before publication.

**Major Comments**

1. The authors measured CTHRC1 expression in twenty paired NSCLC and ANT samples by western blotting, and mentioned that the significant upregulation of CTHRC1 expression was observed in NSCLC tissue. However, they do not show the quantitative data in Fig. 1C. It is indispensable to show the quantification data to refer the statistical significance.

2. The authors used NCI-H1975 and NCI-H2122 cells to examine the role of CTHRC1 in invasion and migration. These H1975 and H2122 cells are harboring EGFR and K-ras mutation, respectively. Please show the rationale for having used these activating mutation-positive cells. According to their data from Supplementary Table 1, the
expression of MMP9, one of key downstream molecule of CTHRC1, seems to be regulated by EGFR mutation status.

3. In the former study that the authors referred as "Reference 22 (Chen YL et al. PLoS One 2013,8:e70324)", Chen et al. reported that knockdown of CTHRC1 decreased the number of adhesive cells to fibronectin-coated plate. On the other hand, significant increase of cell adhesion by depletion of CTHRC1 was observed in this study. While fibronectin-coated plate was used in both studies, the results between the two studies are completely opposite. I suppose that MMP-7 induced by CTHRC1 contributed to degradation of fibronectin, and decreased the adhesion ability of the cells. The authors should clarify the reason of this discrepancy. Did depletion of CTHRC1 affect integrin β1 expression?

4. The authors demonstrated that CTHRC1 and AP-1 complex bound to the promoter region of MMP-7 and MMP-9 by ChiIP assays (Fig. 5 and 6). According to their data, CTHRC1 can translocate to nucleus, however, CTHRC1 has been reported as one of secreted proteins (Pyagay P et al. Circ Res 2005, 96: 261-268. Jiang N et al. Journal of Cancer 2016, 7: 2213-2220.). Actually, the authors measured the serum concentration of CTHRC1 in this study (Fig. 7). Moreover, the authors showed in their previous paper that CTHRC1 was located in cytoplasm by using immunofluorescence (IF) staining (Ke Z et al. Oncotarget 2014, 5. 9410-9424.). Therefore, the authors should show the nuclear translocation of CTHRC1, for example, by western blot with the nuclear protein extracts.

Minor Comments

1. The dot (period) of the end of the sentence in lane 10 of page 17 is not correct.

2. It is very hard to see the band of the ChIP assay in the left panel of Fig. 5F "Region 2". The authors need to show the quantitative PCR data or longer exposure band to recognize more clearly.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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