Author's response to reviews

Title: Differential regulation of MMPs by E2F1, Sp1 and NF-kappaB controls the small cell lung cancer invasive phenotype

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Version: 3
Date: 25 March 2014

Author's response to reviews: see over
Responses to the comments

Manuscript ID: 1759766188103490

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Response to reviewer 1

1 The number (70) of small cell lung cancer specimens used in the study is relatively small.

Response: As suggested, we have made efforts to extend our study via increasing the number of small cell lung cancer patient samples to ensure the results more convinced. Because small cell lung cancer only accounts for about 20-25% of all total lung cancers and the main treatment is chemotherapy and radiotherapy, we collected additional biopsy specimens from 20 small cell lung cancer patients in Jinan Central Hospital. In total, 90 cases were included in the analyses, and results were re-analyzed based on 90 samples in the revised.

2 In the results of “E2F1 was an independent and adverse prognostic factor for SCLC patients” (P. 10), the authors may provide details of the association between E2F1 lower, moderate, and higher expression and clinicopathological variables. Since 67 of 70 of small cell lung cancer showed E2F1 expression, the details of the significant association between E2F1 and clinical stage should be shown.

Response: As suggested, the association of E2F1 expression levels with clinicopathological parameters was analyzed by Spearman’s analysis, and we performed $\chi^2$ test to evaluate the statistical difference between E2F1 lower, moderate,
and higher expression and clinicopathological variables. It should be noted that newly-collected 20 samples were included in statistical analysis. The results showed that high expression of E2F1 was observed in 58 samples from SCLC patients with extensive disease (60), and samples from patients with limited disease displayed weakly-expressed E2F1 (13/30). These results indicated that E2F1 significantly associated with clinical stage (Table 5 in the revised). Based on the results analyzed, we may conclude that E2F1 expression level positively correlated to clinical stage.

3 In multivariate analysis, “E2F1 proved to be an independent and adverse prognosis factor in SCLC” should be revised as “higher E2F1 expression proved to be an independent and adverse prognostic factor in SCLC”.

Response: The point is well taken and revision has been made accordingly.

4 In Figure 3, invasion and migration assay were only performed in E2F1 knockdown cell lines. The authors may use a cell line with low E2F1 expression (eg. A549) to enforce E2F1 expression and repeat invasion and migration assay.

Response: As suggested, A549 cells were transfected with an E2F1 expression plasmid to examine the effect of E2F1 on cell mobility. The results, presented in Additional file 4 in the revised, indicated that overexpression of E2F1 in A549 cells pronouncedly promoted the cell invasion and migration. We have mentioned the results in the text of revision.

5 In the results of “E2F1 significantly inhibited the expression of MMP-9 and -16 in SCLC” (P. 11), the title should be “E2F1 knockdown significantly inhibited the expression of MMP-9 and -16 in SCLC” according to the results.
Response: The point is well taken, and we have corrected the title in the revised.

6 In Figure 4B, present the MMP-3, 7, 9, 14, 15, 16 results of western blotting in the same column according to each cell line will make readers more easily to understand.

Response: The point is well taken, and we have re-organized the results and make them more clearly in the revised.

7 In Figure 6, present 6B in horizontal direction will make readers more easily to understand.

Response: The Fig. 6B has been adjusted as suggested.

8 In “E2F1 modulated Sp1 and p65 expressions in SCLC cells” (P. 13 and Figure 7), present the IHC results of association between MMP-9, Sp1, p65 and E2F1 expression will be helpful to demonstrate their relationship in small cell lung cancer specimens.

Response: The point is well taken. IHC positive staining of MMP-9, Sp1, p65 and E2F1 were 86.67%, 93.33%, 98.89%, and 95.96% in 90 cases of small cell lung cancer specimens, respectively. The detailed results have been added in “E2F1 modulated Sp1 and p65 expressions in SCLC cells”.

9 In Figure 7, present 7C in horizontal direction will make readers more easily to understand.

Response: The Fig. 7C has been adjusted to the horizontal direction as suggested.

Response to reviewer 2:

1 English language should be very carefully attended throughout the text. Some points are hard to read.
**Response:** We have made efforts to carefully read the whole manuscript to avoid of spelling and grammar mistakes. Also, the language of the revised manuscript has been polished by the Edanz Editing professional group.

2 We would like to bring to the attention of the authors on another publication that can be also incorporated in the first paragraph of the Discussion regarding E2F1 status in non-small cell lung cancer (Gorgoulis et al J Pathol 2002).

**Response:** We have included this reference in the revised as suggested.

3 In the results section the reason for choosing to look for E2F1 binding sites in the MMPs should be supported by referencing the work Johnson et al, Cancer Res 2012 (ref 9 in the manuscript).

**Response:** The point is well taken. This reference by Johnson *et al* has demonstrated that E2F1 regulates MMP-9, -14 and -15 expressions via the E2F1 binding sites in the promoters, providing evidence to support our hypothesis that E2F1 is able to bind to MMP-9 or MMP-16 promoter. The reason for choosing to look for E2F1 binding sites in the MMPs based on Johnson’s work has been added. In the results “Sp1 and p65 regulated MMP-9 expression in SCLC cells”, we first tested and verified the function of E2F1 binding site in MMP-9 promoter reported by Johnson, and found that this site predicted by MatInspector software was no function. Then, we found that Sp1 and p65 might regulate the expression of MMP-9. This reference has been cited several times to guide our research.

4 The word bronchial (and not bronchial) should be corrected in figures.

**Response:** The error has been corrected in the revised.