Author’s response to reviews

Title: Extract of Stellera chamaejasme(ESC) inhibits growth and metastasis of human hepatocellular carcinoma via regulating microRNA expression

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Author’s response to reviews:

Dear editor:

Thank you for your information about my manuscript (BCAM-D-17-01157) and reviewer’s points. My responses to the reviewers point to point are as follows:

To reviewer 1:

1. Are the cancer cells used for establishing xenograft model have metastatic potentials?

Yes, hepG2 has the intrahepatic metastatic potentials in our experiment, MHCC-97H is a Highly metastatic hepatocellular carcinoma cell line.

2. How can the authors ascertain the concentration of ESC used will not affect cell viability when studying EMT?

We used MTT method to detect the influence of ESC on cell viability. We choose the low concentration of ESC (little influence on cell viability) to observe the cell migration using transwell assay.

3. Any negative control and positive control when examining the miRNA expression in HCC cells?

The negative control was involved in miRNA expression. (see fig4)

miRNAs were selected to be verified that the miRNA expression in treatment groups was 2 fold changes more/less than(P<0.05) those in control group.
To reviewer 2:

1. The authors lack an explanation of statistical analysis in this study. Figure 1A-B do not show the p-value, and fig. 1C lacks standard deviation of IC50 values.

Fig1A and fig1B were the inhibitory rates of different concentration of ESC. We did not make a comparison between groups, so P-value was not shown.

The standard deviations of IC50 in fig1C were added.

2. The method section lacks important details of many experiments as follows.

2.1 Cell treatment for Immunofluorescence analysis is not described in figure legends as it is written in Line 130. How long were the cells treated with the ESC? And what is the dose of ESC? See Figure 2A

The cells were treated with ESC for 24 h (fig2 legend, line 517-520). The final concentrations of ESC were 50,25ug/ml in HepG2 and MHCC-97H cells (Fig 2A).

2.2 How did the author measure the metastasis tumors and the intrahepatic metastasis rate?

We used the HE and in vivo imaging to detect the metastasis.

2.3 In miRNA expression profile analysis, the author did not provide experiment details of cell treatment such as time duration, concentration of cells.

The experiment details were added （line 151-152）.

2.4 Statistical measurement used in all experiments in this study

Statistical methods was added(line 196-199).

3. The result in Figure 2 B shows that the lowest dose (0.05 ug/mL) of ESC inhibited the cell migration more effective than the highest dose (0.1 ug/mL), yet it clearly contradicts with the result shown in Figure 2C as 0.1 ug/mL of ESC is more effective to increase the expression of E-cadherin and decrease that of vimentin. Explanation of these observation is needed.

I have given the explanation in the discussion(line 273-276).
4. Migration cells should be counted in all fields or five random fields in the same area for unbiased measurement.

I have modified the method (line 114-115).

5. Two cell lines HepG2 and MHCC97H were used in this study, yet the author did not justify their difference and chose detecting the immunofluorescence of vimentin in only HepG2 whereas E-cadherin in MHCC97H.

Because vimentin and E-cadherin expression with our antibody in these two cell lines were different.

6. The author did not discuss at all about the plant exact and its bioactive compounds which has been previously identified. The relevance studies should be cited and explain in discussion.

The relevance study has been cited in introduction (line 58-59).

I must point out that although the composition of Chinese herbal medicine or crude extract is not clear, the single component of traditional Chinese medicine is often not as effective as crude extract and mixture, and cannot represent their role.

7. More information in the legend of Figure 5 is needed.

The legend of Figure 5 was added (line 533-535).

8. In the abstract, changes in gene expression should be clearly stated and "decrease" or "increase".

“Interestingly, only target genes of hsa-miR-107 were changed greatly. ESC downregulated the MCL1, SALL4 and BCL2 gene expression significantly but did not influence the expression of CACNA2D1.”

Sentences above in abstract involved gene expression. MCL1, SALL4 and BCL2 and CACNA2D1 were all the target genes of hsa-miR-107. I think I have stated clearly the changes in gene expression.
9. Are there any pathological lesion in other organs of mice treated with ESC? It would be beneficial to show whether the extract has no toxicity to the animals.

Then the mice were injected intraperitoneally with ESC in different dose according to the MTD (maximum tolerant dosage) experiment. The mice could well tolerate the dose of the ESC. There were no damage to function of liver and kidney.

I think toxicity data are not suitable for the paper.

Please make a comment!

Best regards!

Liu xiaoni