Author's response to reviews

Title: Genistein inhibits tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells

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Author's response to reviews: see over
Referee 1 – from Pan
1. The manuscript needs professional editing for clarity of presentation and English usage.
   Ans: We had send to certify that a medical editor who is a native English speaker associated with MedCom Asia, Inc., for editing for clarity of presentation and English usage.

2. Figure 6, the Western blotting for protein levels should be quantitated with a computer densitometer?
   Ans: We had quantitated with a computer densitometer and level in the figure 6.

3. Figure 6, phosphorylation of IkB is unclear in TPA treated HepG2 cells.
   Ans: We revised the picture of phosphorylation of IkB in TPA treated HepG2 cells.

Referee 2 - from Jiang
1. The references of this manuscript could be updated. For example, the epidemiological introduction of hepatocellular carcinoma could refer to “Jemal A, et al.Global cancer statistics. CA Cancer J Clin. 2011; 61(2): 69-90. ”
   Ans: We updated some references and marked with red. Thank you for your suggestion.

2. In cells invasion assay, the authors calculated the resulting number of invasive cells in HepG2. Please accomplish the quantifications of cells invasion assay in Huh-7, HA22T and BNLCL2.
   Ans: We added the figure of the quantifications of cells invasion assay in Huh-7, HA22T and BNLCL2

3. In Figure 2, Figure 3 and Figure 4, “**” means p<0.01 compared to untreated cells. What does “untreated cells” mean? control, mock or TPA-treated cells?
   Ans: Sorry, description is not clear. We will revised those sentence.

4. In Figure 5 A and C, the authors intent to show the inhibitory effect of Genisten on TPA-activated transcription of NF-kB and AP-1. As shown in the Figures, the density of the band of the second line (the concentration of Genisten is 0µM) is undoubtedly much lighter than the density of the band of the third, the forth, even the fifth line (the concentration of Genisten is 5, 10
and 20µM). Does Genisten of 5, 10, and 20µM activate the transcription of NF-kB and AP-1?

Ans: This is mislabeled. We re-do this experiment and provide new figure.

5. In the text, the cells were treated with TPA, with or without Genisten, for 20 h, and promoter activity was measured by a luciferase assay. However, the figure legend of Figure 4 shows that the cells were treated with TPA for 16 h. Which one is right?

Ans: We rechecked the procedure that "16h" is the correct point in time. We revised the sentence in the text.

6. The second sentence in the figure legend of Figure 5 shows that EMSAs of nuclear extracts of cells were performed after 24h. However, the forth sentence in this figure legend shows that cells were pre-treated with Genisten with or without inhibitors for 30 min before incubation with TPA for 45 min, then nuclear extracts were prepared. Which one is right?

Ans: We rechecked the procedure that "45 min" is the correct point in time. We revised the sentence in the text.

7. Figure 5 and Figure 6A show the effect of Genisten on TPA-activated transcription of MMP-9, NF-kB and AP-1, why not combine the two Figures?

Ans: We merge the two Figure Figure 5A and 5B