Reviewer’s report

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

Version: 2 Date: 26 April 2013

Reviewer: Hongchi Jiang

Reviewer’s report:

In the manuscript ‘Genistein inhibits tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells’ by Shulhn-Der Wang et al, the authors demonstrated the inhibitory effect of Genistein on tumor invasion in human hepatocellular carcinoma cells in vitro. The results indicate that Genistein inhibits TPA-induced expression and activity of MMP-9 by inhibiting AP-1 and NF-kB activity. Furthermore, the authors also demonstrated that the inhibitory effect of Genistein on TPA-induced activation of MAPKs, PI3K, and Akt/protein kinase B. Overall, this is an interesting study and for the most part the experiments have been well performed with a sufficient number of experimental approaches. However, there are a few crucial questions that should be addressed or revised.

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.”

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.

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?

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?

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?

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 inhibitos for 30 min before incubation with TPA for 45 min, then nuclear extracts were prepared. Which one is right?
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?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have no competing interest in relation to this paper to declare.