Reviewer's report

Title: Baicalein mediates inhibition of motility and invasiveness of skin carcinoma through Ezrin in A431 cells

Version: 2 Date: 3 October 2011

Reviewer: Pei-Wen Hsiao

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This study tested one direct hypothesis whether baicalein can regulate ezrin function and thus the cell migration and invasion in vitro. The demonstration of baicalein effect on cellular expression of ezrin is novel, straightforward and convincing. However, the entire study is restricted in cell culture. Therefore, the title is claimed beyond the evidences. More appropriate title is suggested as “Baicalein inhibits the cellular motility and invasion through Ezrin in human epidermoid carcinoma A431 cells”. It should be noted that A431 cell line is composed of malignant keratinocytes derived from a vulval epidermoid carcinoma and more relevant to squamous cell carcinoma rather than other skin cancers, such as basal cell carcinoma and melanoma. Moreover, there are several scientific problems (listed below) misrepresented in the manuscript and need to be corrected.

1. Figure 1A, baicalein is the aglycone compound of baicaline.

Data of Fig.1B use relative levels of MTT assay. The baseline level used as 1 is not indicated. The statistics using Student’s t-test is a paired comparison. What has been compared with the dose-effect of 50µM and 60 µM baicalein is not indicated, either. Number of independent experiments is not indicated in Figure 1.

2. The relative expression of Ezrin and phos-Ezrin at Thr-567 were determined quantitatively to show the dose-effect relationship of baicalein in A431 cells. Since each antibody has a distinct titer from other antibodies, it is more appropriated to plot the #-actin normalized dose-effect using vehicle control as 1 (baseline level). Although different antibodies have their own different baseline, they can still be plotted side-by-side and compared for the tendency of alteration.

3. Page 12, line 9-10, the statement “The crawling movement of cells transfected with Ezrin si-RNA was also inhibited by baicalein treatment (Fig. 4-c).” is wrong. The result showed that cells treated with baicalein reduced the cell motility similar to the effect of knocking down Ezrin in A431 cells.

4. Figure legend of 5 indicates wound-healing assays were used to detect cell migration. I guess it was a transwell migration assay in a Boyden Chamber on an uncoated membrane filters. Ensuing legend of the entire 5A is wrong. Y-axis of all bar graphs in Figure 5 reflects a measure of cells/field. The statement in the last line of page 12 “only 27% motility left” is not reflect the results shown in the
5. Page 13, line 12-14, “si-RNA Ezrin-A431 cells were then transiently transfected with pcDNA3.1 ...” in the figure 6, suggests that baicalein compromised cell motility and invasion via decresing ezrin expression and protein function. The degradation of Ezrin mutant is also seen in figure 6A (lane 3 vs. 4), suggesting that protein degradation of Ezrin in addition to its transcriptional regulation is also involved in the loss of Ezrin function. This should be discussed in first paragraph of page 15.

5. Page 15, line 12-14, the mechanism that
Page 15, line 12-14, “baicalein decrease cell motility and invasion of A431 cells transfected with a si-RNA construct’ does not reflect the results shown in Figure 6B and 6C.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.