Reviewer's report

Title: Inhibition of Raf-MEK-ERK and Hypoxia pathways by Phyllanthus Prevents Metastasis in Human Lung (A549) Cancer Cell Line

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Reviewer: Pei-Li Yao

Reviewer's report:

The revised manuscript entitled “Inhibition of Raf-MEK-ERK and Hypoxia pathways by Phyllanthus Prevents Metastasis in Human Lung (A549) Cancer Cell Line” had been modified in response to reviewer’s comments. In general, the reviewer appreciates and is satisfied with the majority of responses that authors provided. Few questions still remain and need to be addressed.

Major Compulsory Revisions

1. The authors demonstrated the significance of 2D-western blot in this study (Figure 3A-I); however, 1D-western blot was also performed to detect VEGF level (Figure 3J). Why did the authors use two approaches? Although the colorimetric detection method is relatively less sensitive than other detection methods, using 150 micrograms of each protein in 1D-western blot is too much (Page 10, line 206). The reviewer understands no suitable control for 2D-western blot, while a proper loading control for 1D-western blot is necessary.

2. FUSE-binding protein (FBP) binds to FUSE-element in c-Myc promoter, further regulating c-Myc expression. This binding interaction during transcription regulation is not sufficient to explain the presence of FBP in the blot without incubating with FBP-antibody, especially under a fully denatured condition.

Minor Essential Revisions

1. Zymogens can be activated by the processes of denaturation (SDS) and renaturation (Triton X-100); therefore, proMMP2 and proMMP9 should be visualized in the zymogram. In fact, the advantage of using zymography is to detect both pro- and active forms of MMPs.

2. In Abstract, the authors mentioned that MMP2, MMP7 and MMP9 are down-regulated by Phyllanthus extracts according to proteomic analysis (lines 44-46). However, these three proteins were not present in tables 2 and 3. That’s why the reviewer asked if the authors detect major changes in these three proteins in proteomic results. Again, the authors responded to the reviewer, confirming that no changes had been found. It is suggested to modify the description in Abstract.

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 declare that I have no competing interests.