Reviewer's report

Title: The natural compound Guttiferone F sensitizes prostate cancer to starvation induced apoptosis via calcium and JNK elevation

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Reviewer: Wen-Xing Ding

Reviewer's report:

Authors investigated the beneficial effect of a novel natural compound Guttiferone F (GF) against prostate cancer. The authors utilized both in vitro and in vivo and a series comprehensive cellular and biochemical assays to evaluate the anti-cancer effects of GF. Mechanistically, the further demonstrated that GF treatment in combination of growth factor withdrawal increased cellular Ca2+ surge, mitochondrial morphological changes and JNK activation. More importantly, GF significantly suppressed tumor growth in a xenograft model. This is a very interesting and novel study, which may offer novel therapeutic approach for treating cancer. Experiments were generally well conducted and properly controlled. The manuscript is easy to read and follow. However, the studies were a little bit descriptive in nature and could be further improved by applying some pharmacological approaches.

Major Concerns:
1. While they authors showed GF treatment with growth facto withdrawal increased intracellular Ca2+ surge and JNK activation, but these findings only established an association with GF-induced apoptosis not the causal effects. It would be nice to use calcium chelators such as BAPTA-AM and JNK inhibitors to further explore whether inhibition of either calcium increase or JNK activation would attenuate GF-induced apoptosis.

2. Figure 3A was not convincing. It is well known that Mitotracker red is not suitable for monitoring mitochondrial membrane potential. Although the mitochondrial uptake of Mitotracker red is dependent on mitochondrial membrane potential, once mitochondria are stained, Mitotracker Red covalently binds with mitochondrial proteins and is not released even after the depolarization of membrane potential. Therefore, mitotracker red is mainly used to monitor mitochondrial mass or morphology but is not suitable for mitochondrial membrane potential. Tetramethylrhodamine methyl ester (TMRM), and tetramethylrhodamine ethyl ester (TMRE) should be used to monitor mitochondrial membrane potential changes after GF treatment.

Minor concerns:
1. PC3 cells are known p53 deficient. This could be part of the reason why PC3 cells were more resistant to GF treatment compared with LNCaP cells. This should be discussed.
2. Why Table 1 has two LNCaP cells that have two different IC50?
3. Figure 7 needs to indicate what statistical methods were used?
4. There were many grammatical errors in the manuscript. This reviewer would suggest the authors to seek some help from English native speaking person or some English writing professionals.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

No conflict of interest.