Reviewer's report

**Title:** Chemopreventive effects of berberine on intestinal tumor development in APCmin/+ mice

**Version:** 2  **Date:** 20 May 2013

**Reviewer:** Kshipra Singh

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In this manuscript, Cao et al., tested the chemopreventive effects of berberine on intestinal tumor development in APCmin/+ mice. The authors found that total number of intestinal tumors in APCmin/+ mice were reduced after 12 week of berberine treatment and showed that berberine inhibits β-catenin and EGFR activation and also suppress COX2 and PGE2 expression. They also found that, when APCmin/+ mice were treated with berberine the PCNA positive cells were significantly reduced compared to the control (untreated) group. Further, the authors showed that the berberine treatment increases apoptotic cells.

The aim of this study is to address the effect of berberine against intestinal tumor in APCmin/+ mice. Though the data presented here are somewhat convincing, but proper controls and plenty of data is missing, which raises many concerns.

**Major Compulsory Revisions**

1. Could the authors show the gross appearance of the intestinal tumors from untreated and berberine treated APCmin/+ mice? In Table-1 and Fig-1, total numbers of intestinal tumors are not shown, which is confusing specially when in results the main focus was on total number of intestinal tumors. Author should show one extra column in table with total number of tumors?

2. In Fig.3C, the authors used tumor lysates to perform western blot. In methods it is unclear how lysates and nuclear fractions were made. Authors should show β-catenin in cytoplasm, because accumulation of β-catenin in cytoplasm leads to its translocation in nucleus, It is important to know if berberine only inhibiting β-catenin translocation in to the nucleus or berberine enhancing β-catenin degradation.

   It is inappropriate to use Ki-67 as nuclear protein loading control because it is a cell proliferation marker and in results it is said that Ki-67 was reduced after berberine treatment while data was not shown? It is very mystifying.

   Could it be possible to use some other nuclear protein loading control like fibrillarin, and show the densitometry? Author should also show total EGFR. It is also important to compare tumor vs non-tumor area for all these studies in both untreated and berberine-treated groups.

3. In Fig.4, Could it be possible to show the densitometry for western blot?
4. Authors claimed less Ki-67, Cyclin D1, less p-ERK and less p-Akt, in Results and Discussion but data is not shown in Figures. It is very important and essential to show these data as Figures?

Minor Essential Revisions
Use of word "control" in whole manuscript is very complicating; while control is actually untreated APCmin/+ mice.
Method should be written in more detail.

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

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

**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