Reviewer's report

Title: The involvement of AMPK/GSK3-beta signals in the control of metastasis and proliferation in hepato-carcinoma cells treated with anthocyanins extracted from Korea wild berry Meoru

Version: 4 Date: 24 August 2013

Reviewer: Nitin Telang

Reviewer's report:

Major Compulsory Revisions:

Background:

Line 8: “Several studies----over-activation of β-Catenin”

This statement as written fosters confusion. Evidence in the literature suggests that the role of β-Catenin in tumorigenesis is dependent either on the presence of mutation or decreased cytosolic degradation of normal β-Catenin, in part due to aberrant Wnt/APC signaling pathway. The original statement therefore, needs to be revised to include additional published evidence that is specific for the role of over-activation of β-Catenin in tumorigenesis or tumor metastasis.

Methods: The description of the isolation procedure for anthocyanins is adequate. However, it is not clear whether anthocyanin glucosides are the only components present in the extract. Therefore, detailed procedure for the quantitation of specific anthocyanin glucosides and/or other constituents present in the extract need to be included in the Methods Section. Additionally, the results from the quantitation assay should be included in the Result Section of the manuscript.

Results:

1. Growth Inhibition by Anthocyanins: The statement that in vitro growth inhibition (cytostatic growth arrest and cellular apoptosis) represents chemoprevention is confusing since tumor derived Hep3B cells are used for both in vitro and in vivo assays. These data may be more appropriately described as in vitro and in vivo effects on tumor cell phenotype.

2. Based on the quantitation of Anthocyanin glucosides present in the extract, it needs to be clarified whether the doses used represent total anthocyanin or a specific glucoside.

3. Fig. 1 B, 1C: The dose response data for Anthocyanins exhibits a modest inhibition cell survival (80% of control) at 200 µg/mL and about 40% of control at 400 µg/mL (Fig. 1B), while the effect of Anthocyanins on apoptosis is presented at 100 and 200 µg/mL doses (Fig. 1C). What is the status of apoptosis at the higher effective dose of 400µg/mL?

4. Data on AMPK Si RNA and Compound C: The results from the Si RNA experiments are convincing. However, the experiments with the chemical
inhibitor of AMPK have generated distinct data. The interpretation for the observed differences by the two approaches needs to be included.

Discussion:

1. The characteristic effects of AMPK Si RNA and Compound C should be discussed in greater detail in the discussion section.

Minor Essential Revisions:

Results:

1. p. 10, Line 5: “but this effect----co-treatment with Anthocyanin” This statement as written is confusing. The data suggests that co-treatment with Anthocyanin inhibits the effect of IGF-1. This aspect needs to be clarified.

2. p 10, Lines 8-9: “To exclude----- (Fig. C)” This sentence is incomplete and therefore, is confusing. This sentence needs to be revised for clarity.

Discussion:

Line 4: “attenuated transcription oncogenes”. This part of the sentence needs to be expanded to specify oncogenes whose transcription is affected by β-Catenin.

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