Reviewer's report

Title: Benzyl butyl phthalate induces migration, invasion, and angiogenesis of Huh7 hepatocellular carcinoma cells through nongenomic AhR/G-protein signaling

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Reviewer: Rituraj Konwar

Reviewer's report:

In this manuscript, the authors documented significant amount of work demonstrating that benzyl butyl phthalate promotes hepatocellular carcinoma cells and their metastasis under in-vitro and in-vivo experimental conditions. I have the following comments on this manuscript.

Major Compulsory Revisions:

Abstract:
# Background: “a novel non-genomic AhR mechanism”, was this pathway novel or information that BBP acts through this pathway in new.

Background:
# Can be improved and made more specific to current investigation of BPP and AhR. Citations on prior report of nature of BBP and AhR binding or interaction can be incorporated, if any.
# “Accumulating evidences showed the effect on liver induced by phthalates”. Incomplete sentence, which or what effect? Can be deleted if not necessary.
# “Hepatocellular carcinoma” is more preferred over old terminology of “malignant hepatoma” which many uses simply as “hepatoma” and gives impression of benign tumor.
# “......treated...Huh7 and PLC cells with BBP and the in vivo cancer growth were evaluated”. Only in vitro studies in PLC are reported and only in supplementary results.
# Author mentions that earlier reports suggests AhR/HDAC6/c-Myc and other signaling pathway for promoting invasiveness of cancer by phthalates, how or why author selected non-genomic signaling or AhR/G protein pathway for this study can be incorporated.

Methods:
# Authors discuss earlier reports on HepG2 cells in the background section. But used Huh7 and PLC cells. Are there any criteria adopted for choosing liver cancer cell lines, if so please mention.
# 500 mg/kg of BBP intra peritoneal injection to promote metastasis, why this dose and route was selected should be mentioned.
# Animal studies, intrahepatic xenograft experiments are not described adequately. Groups and numbers in each group, definition of control used, whether it is no Huh7 cells or HuH7-IFP cells or HuH7-IFP cells with BPP, description or citation of surgical procedure, how distant metastasis was determined etc. If, any threshold or cutoff used for considering metastasis positive organ. Were there any differences in histo-pathological characteristics of BPP promoted tumor versus BPP non-treated tumors can be included.

Discussion:
# PLC results were not discussed, and also, why probably BPP is not effective.

Conclusions:
# In methodology, authors have described use of ERK antibodies, they have used ERK inhibitor in some experiments, and there are no results on ERK phosphorylation with BPP treated in either Huh7/PLC/HUVEC cells. But, concludes “BBP promotes angiogenesis via the AhR/ERK/VEGF pathway”, also in “Abstract” section. ERK phosphorylation experiments can be included.

Figures:
# Figure 1 legend: “Data shown in the upper image are” can be replaced with “Agarose gel image is”? Also can be expanded as “Each value in the graph <obtained through denisitometry> is the mean…” . Also, reorganize “BBP (1 µM) or DMSO for 15minutes as control group”
# Though supported by GFP intensity graph, Figure 2 A and B image and scale resolutions are poor. Correct “magnificent x600”
# Figure 2 D: Whether upper left image is of BPP untreated cells? Localization of AhR and G-alpha in BPP untreated would have been interesting. Are the two lower images having different significance here?
# Figure 3 legend: 3A is missing. “BBP casued the cleaveage of PIP2 …..”. Apart from the spelling mistakes, if description needs to be exact to the figure, it should be “reduction of level of PIP2” rather than “cleavage”.
# Figure 3 B legend: “Real-time imaging of calcium … by Cell-R microscopy” Please include of what and in which condition? Also, description of vertical axis in Figure B, C & D can be provided in legend.
# Text of figure 3 B& C not clear due to low resolution. I feel, even B & C can be clubbed.
# Figure 4C legend: If it is time points of post-BPP treatment, please incorporate.
# Figure 4 legend: “β-actin was used as an internal control. Move from description of “4E” legend to be part of “4D” legend of Akt-phopho detection image.
# Figure 5B: Upper and lower images should be marked with time points.
# Figure 5 B: correct vertical axis title “invated cells/field”
# Figure 5 D & E legend. Mention control if it is BPP untreated or vehicle treated
tumor group.

# Supplementary figure: It should be described in result or discussion, instead of background.

Minor essential revisions:
Lacks page numbers, make difficult to specify comments on the manuscript. The manuscript also uses lots of abbreviations, full form of which should be incorporated at the time of first use. eg. DEHP etc. Also, giving full forms of abbreviations in abstract can be helpful. In addition, few spelling and grammatical mistakes needs to be corrected.

Discretionary Revisions:
# Keywords: Also add hepatocellular carcinoma or Huh7
# Conclusions: “… useful for developing approaches to prevent and treat liver cancer”. Please remove and give prospect of immediate impact.

Level of interest: An article of importance in its field

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