Reviewer's report

Title: Inhibition of DNA methyltransferase activity and expression by treatment with the pan-deacetylase inhibitor panobinostat in hepatocellular carcinoma cell lines

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Reviewer: Huanjie Shao

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In the manuscript, Zopf et al. investigated the effects of pan-DACi panobinostat on the activity and expression of DNA methyltransferase (DNMT) in HepG2 and Hep3B cells in vitro and HepG2 xenograft model in vivo. The authors observed a rapid inhibition of total DNMT activity after treatment with panobinostat, while the inhibition of genes expression was at later time points. Along with the suppression of DNMT activity and expression, the methylation status of hypermethylated genes, like APC, was decreased as well, followed by the re-expression of APC mRNA. Although the study is interesting, some evidences are not convincing. And the conclusion seems to be overstated. There is no direct evidence to show that the rapid inactivation of DNMT activity by treatment of panobinostat is due to posttranslational modification, like protein folding and acetylation status; and thus the conclusion seems to be a hypothesis rather than a conclusion drawn from the results.

Major compulsory revisions

1. Treatment with panobinostat resulted in a rapid and significant decrease in DNMT activity by more than 46% after 6 h of treatment even when there are no changes in DNMT protein levels. However, at later time points, like 72h, the protein levels of DNMTs was significantly reduced, even down to zero (DNMT1 in HepG2 cells); the DNMT activity was still kept similar inhibition around 40%. Is DNMT activity independent of DNMT expression? Or the authors want to elucidate that the rapid inactivation of DNMT activity interfered by panobinostat directly and the direct interfering only lasted for a short time period, while inhibition of DNMT activity after 24h was mainly/only due to the reduced protein levels? If so, the authors need to show more evidences.

2. Figure 2, blots of DNMT3b is in poor quality. And some densitometry values seem not to be unanimous with the bands. For example, the blot of DNMT3a in Hep3B cells with panobinostat 24 h is obviously weaker than its control, but the densitometry value is 1.3.

3. Obviously, RASSF1A is not a good evidence to support the effects of panobinostat on methylation and re-expression of target gene, because its response to panobinostat is quite weak. Did the authors check the status of other known hypermethylated genes, e.g. RAR#, CDNK2A, GSP1 or CHD13?

4. APC methylation was quickly and significantly suppressed after 6 h treatment
of panobinostat in Hep3B cells. DNA methylation is thought to be relatively stable when compared with other epigenetic modification. DNA demethylation caused by DNMT activity inhibition is a passive process and needs longer time for DNA replication and cell division. Can the author explain whether there are any active DNA demethylation involved after treatment of panobinostat and by which resulted in a rapid suppression of APC methylation?

5. Figure 6 is a hypothesis only partially supported by this work. The authors need more evidences to prove it.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests