Reviewer’s report

Title: Increased phosphorylation of histone H3 at serine 10 is involved in Epstein-Barr virus latent membrane protein-1-induced carcinogenesis of nasopharyngeal carcinoma

Version: 2 Date: 23 January 2013

Reviewer: Chang-Han Chen

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The study by Li et al explores the relationship between phosphorylation of Histone H3 and LMP-1-elicited tumorigenic behaviors of nasopharyngeal carcinomas. Strength of the study lies in the association that linked up-regulated p-H3Ser10 was positively correlated with the LMP-1 in nasopharyngeal carcinomas. Elevated LMP-1 expression enhanced the cell proliferation and foci formation through p-H3Ser10 modulation. However, the authors extend these observations by demonstrating the coupling of LMP-1 to the activation and phosphorylation of MSK1 to induce cell proliferation. Overall, this is a concise, and well-designed that can be further improved by incorporation of additional experimentation and textual clarifications. These and other comments are listed below:

1. The author demonstrated that the LMP-1 expression was located on cell membrane and cytoplasm (Figure 1D); however, in the figure legend D, it was illustrated that the negative expression of LMP-1 in NPC tissues. There is not consistent between the context and figures (or figure legends).
2. In the context, there are not any descriptions about figure 1E, and additional file 1.
3. In figure 1, it is not clear to observe the results of p-H3 and LMP-1 IHC staining in NPC tissues. The author should magnify the figures.
4. In figure 1F (for negative control), the primary antibody was replaced by PBS to perform the IHC staining. The author should use peptide competition approach to identify the specificity of primary antibody.
5. The figure numbers labeled in the context were wrong. The figure 2A to B should replace to figure 3A to C in the following paragraph “Phosphorylation of histone H3…..”.
6. Silencing of the histone H3 and MSK1 gene in these cell lines should be confirmed by Q-RT-PCR and Western blotting.
7. In figure 3B and C, the author co-transfected with LMP-1 and wild type histone H3 (or mutant histone H3; siH3) in CNE1 cells to address whether phosphorylation of histone H3 at Ser10 could regulate the LMP-1-induced cell transformation. The question is how the authors knew that these colonies which grew on the soft agar had expressed the LMP-1 or histone H3 (or mutant form) or
both.

8. In the discussion section, the following sentence should be added the reference “Recent studies showed that histone H3, especially the Ser10 motif, has oncogenic effects and....”.

9. The cut-off for positive and negative scores (# 5% or < 5%) of LMP-1 expression seems to be arbitrary. Why author used this grading score for analyzing the LMP-1 expression by IHC? Please describe it in the material and method section.

10. The siRNA sequences of histone H3 and MSK1 should be describe in the Material and Method section.

Major Compulsory Revisions

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.