Review of: "Interaction with TopBP1 mediates human papillomavirus 16 E2 plasmid segregation/retention function and stability during the viral life cycle"

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Title: Interaction with TopBP1 mediates human papillomavirus 16 E2 plasmid segregation/retention function and stability during the viral life cycle.

Comments for authors:

In this manuscript, Prabhakar et al investigated the requirement of TopBP1 interaction for HPV16 E2-mediated segregation of HPV episomal DNA during mitosis.

Authors established a novel luciferase-based segregation/retention assay and the results nicely demonstrated the importance of the interaction with TopBP1 for HPV16 E2 to contribute to the plasmid segregation during HPV16 lifecycle.

This study provides a significant contribution to the understanding of HPV E2 protein functions as a segregation/retention factor since there has been little evidence while it has been suggested by the interaction of E2 with mitotic chromatin.

Major critique:

1. Figure 2

Was the methodological integrity of novel luciferase-based segregation/retention assay validated?: The direct evidence would be needed to show that the luciferase activity corresponds to the quantity of plasmid DNA presence but not to the transactivation of luciferase gene by E2 interacting with TopBP1. For example, FISH at the day 3, 6 and 9 after the transfection.

Minor critique:

1. p10, line 207

The expression level of E2 protein in HFK+HPV16 cells was increased in the presence of 3T3-J2 fibroblasts (Figure 6A lane 2 and 3). At the same time, Cyclin B1 level was increased in the presence of 3T3-J2. Does this suggest the increased cell proliferation in the presence of 3T3-J2 might have resulted in increased HPV16 DNA copy numbers thereby the expression of E2 protein level might have been increased?

2. p15, Materials and methods: “Generation of fluorescently tagged plasmids and transfection”. What is the method of fixation?

3. TYPO: p19, line 423: Milion3T3.