Reviewer's report

Title: Human papillomavirus is found in peripheral blood CD20+ and CD56+ cells during HPV-16 semen infection.

Version: 1  Date: 7 November 2013

Reviewer: Winnie Tong

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This is an interesting study that neatly demonstrates the presence of HPV16 E6 and L1 proteins co-localised with HPV16-DNA in circulating and semen lymphocytes. Traditionally, all papillomaviruses are considered strictly epitheliotropic.

Major Compulsory Revisions

1. Title – insert “proteins” after “papillomavirus”; insert “and semen” after “blood”.

2. Methods – Patients - lines 8-10 and 14-15 – please clarify how men were divided into “HPV-DNA positive” and control groups. Did men have to be HPV-DNA positive in semen (or sperm?) by FISH, AND be HPV-16 positive on whole semen to be included in the “HPV-DNA positive” group? Which genito-urinary sites were screened to determine controls were truly HPV-DNA negative? If group selection was determined by HPV16-DNA in sperm and/or HPV16 in non-sperm cells in semen, then p-values should not be applied to the last two columns of Table 1.

3. Methods – FISH for HPV-DNA – were positive controls done?

4. Methods – Immunofluorescence – were positive controls done? Given the emphasis placed in this paper on HPV16 proteins in CD20+ and CD56+ cells, positive controls for all leukocyte markers used should be shown.

5. Figure 1a I and II – please show corresponding light microscopy or phase contrast images to accompany the FISH images, to illustrate the morphology of the cells described in lines 3-6 under Results.

6. Results – Analysis of semen round cells – lines 21-24 – please clarify if >95% of infected round cells were CD45 positive or negative.

7. Results – Analysis of semen round cells – lines 24-27 – are CD4 and/or CD8 T-cells found in semen? Of semen round cells, what proportions are CD4/CD8/CD20/CD56 positive (regardless of HPV positivity)? What percentage of CD20+ or CD56+ semen round cells express HPV16 E6 or L1 in the HPV16-infected group?

8. Results – Analysis of peripheral blood leukocytes – what percentage of CD20+ or CD56+ PBMCs express HPV16 E6 or L1 in the 4 men who are PBMC FISH+?
9. Conclusions – line 25-27 – please reference statement regarding NK and iNKT cells being the main innate cells responsible for clearance of HPV infection (otherwise remove).

10. Conclusions – could the authors expand and further emphasise that the detection of HPV16 proteins in these cells do not necessarily indicate productive infection. Therefore please remove the second last sentence of the abstract conclusion.

Minor Essential Revisions

1. Table 2 – remove unnecessary abbreviations from the legend.

2. Conclusions – line 17 (second last page) & 22 (last page) – change ‘rise’ to ‘raise’.

Discretionary Revisions

1. Conclusions – lines 8-11 (last page) – an alternative explanation for these findings is that semen immune cells can become unproductively infected with HPV16 when trafficking through the seminal compartment in HPV16-infected men, and these cells may traffic back into circulation and be detected at low frequency in peripheral blood.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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