Author's response to reviews

Title: Cell-cycle and Suppressor Proteins Expression in Uterine Cervix in HIV/HPV co-infection: Comparative study by Tissue Micro-Array (TMA)

Authors:

   Alcina F Nicol (nicol@ioc.fiocruz.br)
   Andreia Pires (andreapires@fontemd.com.br)
   Simone R Souza (simone@fontemd.com.br)
   Gerard J Nuovo (nuovo-1@medctr.osu.edu)
   Beatriz Grinsztejn (gbeatriz@unisys.com.br)
   Aparecida TristAGBPo (aptristao@iff.fiocruz.br)
   Fabio B Russomano (frussomano@superig.com.br)
   Luciane Velasque (velasque@fiocruz.br)
   Jose R Lapa e Silva (jrlapa.ntg@terra.com.br)
   Claude Pirmez (pirmez@ioc.fiocruz.br)

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Author's response to reviews:


To the Editor-in-Chief

Dear Editor,

Once more we would like to thank you for the kind and helpful comments regarding our manuscript entitled “CELL-CYCLE AND SUPPRESSOR PROTEINS EXPRESSION IN UTERINE CERVIX IN HIV/HPV CO-INFECTION: COMPARATIVE STUDY BY TISSUE MICRO-ARRAY (TMA)”. Please find attached the answers to the questions raised by the reviewers.

Yours sincerely,

Alcina Nicol
nicol@ioc.fiocruz.br

Reviewer: Nicolas Wentzensen

The authors claim that there are significant differences of marker expression between HPV+/HIV- and HPV+/HIV+ women. However, as mentioned in the previous review, the data in table 1 does not account for lesion severity. The HPV+/HIV- group contains a number of cancers, while the other group only includes CIN. The high-grade/low grade rations between both groups are different. Therefore, table 1 is maybe very misleading if the comparison is not adjusted for disease status. You need to show a horizontal comparison of the cells in tables 2 and 3.
Answer: Table 1 now shows, as recommended by the reviewer, a compilation of the data for VEGF, p27, Rb, and Elf-1 expression relative to lesion severity (CIN I versus CIN II/III) for the two groups. On table 1, we could find statistical differences in the VEGF and P27 expression in the Low grade and high grade CIN in the HPV+/HIV+ co-infected group compared to only HPV infection, however Rb and Elf-1 was not significant.

We decided to group CIN II and CIN III as this is the norm in most studies, including the influential Bethesda system, which considers each HGSIL/HGCIN.

While chronic cervicitis may be included in table 2 as a comparison, it is not appropriate to put it into table 3 with the headline “HPV+/HIV+”. Either you need to use normal tissue/cervicitis from HPV+/HIV+ women, or omit this.

Answer: We agree that chronic cervicitis (in women without HPV or HIV infection) is the logical control to our viral infected groups. We have eliminated Table 3, since the CIN I versus CIN II/III data for HPV+/HIV+ women is in Table 1.

You need to give better a priori hypotheses for statements like: “We hypothesize that regulatory and cell cycle protein expression might be modified in the cervix of HIV/HPV co-infected women under HIV-influence”.

Answer: We thank you, your suggestion, however we’d like to maintain our hypothesis, since it was based on previous reports that regulatory and cell cycle expression might be modified by HIV influence; we then assayed the present markers, since they are involved in HPV-associated CIN progression.

The question on candidate selection is directed at the analyzed markers, not the analyzed tissues. How were these markers chosen? This needs to be elaborated in the introduction. It seems strange that in the introduction it is hypothesized that exactly these markers are modified by HPV/HIV coinfections, despite there are other important markers that have not been analyzed in this study. There must be some a priori rationale for picking these markers.

The reviewer raised good points. We agree that many different markers could have been chosen. We selected several based on their reported roles with HPV infection (Rb), angiogenesis (VEGF, Elf-1) and, most importantly, on their possible roles in facilitating invasive cancer either via interference with tumor suppressor genes or enhancing new blood vessel formation. The choice for each analyzed markers was explained and included on pages 3 and 4, background section. On this section we showed a priori rationale for picking these markers.

Minor Essential Revisions

The statement “Increasing grades of CIN were associated with decreased Rb epithelium expression in HPV+/HIV-negative infected cervices (average of cells/mm2 in CIN I: 17.9, CIN II/III: 4.8, and tumor 3.9, p =0.003), but not in cervices of HPV/HIV co-infected women (average of cells/mm2 in CIN I: 28.9 and CIN II/III: 10, p= 0.081). “ is not correct. There is a decrease, but it was not found to be significant.
We fully agree with the reviewer, and the text was modified.

HPV30 and HPV70 should not be listed as HR types

We are grateful for this observation and HPV30 and HPV70 were deleted from the list as HR types, on page 6, HPV type section.

Wording throughout needs to be improved

*example:
- “did not produce statistical differences”
- “there was strong statistical significance (p<0.05)”
- IQR=Interquartile range, not interval

Accordingly the reviewer’s suggestion, changes were made to:

- did not find statistical differences (pg 10 last paragraph)
- there was a statistical significance (p<0.05).
- IQR was corrected (on tables 1,2 and 3)

Reviewer: Raphael P Viscidi

We had the entire paper revised by a person with expertise in both molecular pathology and English grammar (at the Ohio State University - USA).