Author's response to reviews

Title: Detection of human papillomavirus DNA and p53 codon 72 polymorphism in prostate carcinomas of patients from Argentina

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Author's response to reviews: see over
Dear Dr. Newmark,

We have now revised our manuscript according to the very helpful suggestions of the referees.

In the following please find our point-by-point response.

Response to reviewer L. L. Villa:

Major compulsory revisions:

1. “Authors must provide an explanation why numbers of cases do not match in the different tables”.
   Response: The studied population includes only those patients from which the isolated DNA samples could be used for PCR assays, i.e. the beta-globin test was positive. However, the amount of DNA isolated was very low in several cases. Therefore not all PCR assays could be performed with all DNA samples. This problem and the selection of samples for the PCR assays are now explicitly stated in the Materials & Methods section, in all 3 parts dealing with DNA isolation and PCR.

2. “Was DNA isolated from blood as efficiently amplified as from fixed tissues?”
   Response: Yes, there were no gross differences.

3. “How did the authors exclude false negative results in the HPV consensus primer PCR that generates a fragment often too large for specimens containing damaged DNA?”
   Response: We did not explicitly exclude such possibility. However, the number of HPV-positive samples in the carcinoma group is high thus indicating that the isolated DNA was of sufficient quality to amplify a 450 bp region.

4. “Even more surprising is the lack of HPV typing due to insufficient DNA”.
   Response: It may be surprising but nevertheless it was the reality. The small paraffin-embedded tissues from the hospital archive could not be used for research again according to legal disposition.

5. “Information about the microdissection procedure is lacking”.
   Response: Detailed information about the microdissection procedure is given in Materials & Methods in the part entitled “Dissection of neoplastic tissue”.

6. “…second assay to define p53 codon 72 polymorphism: a full description is included on page 7, but the results are not shown”.
Response: We show the results of the allele-specific PCR in Figure 2 for 20 out of 87 analyzed DNAs. Because the second assay gave a full confirmation of the results of the first assay, we decided not to show the confirming results in addition.

7. “Results presented in Figure 1 need further explanation”.

Response: We have modified Figure 1 by presenting only the results of the filter hybridization. The appearance of two hybridization signals is explained in the legend. In the first version of the Figure there was a mistake concerning the size marker, the corrected version now shows the position of the 450 bp fragment.

Minor essential revisions:

8. The term “HPV infected” has been changed into “HPV-positive” throughout the text.

9. Figure 2: in our opinion Figure 2 is informative because it demonstrates the unambiguous clarity of the allele-specific PCR assay. We have revised the legend in order to explain all definitions used in the Figure.

Response to reviewer A.M. Garcia-Carranca

Minor essential revisions:

1. All measures taken to avoid contamination of the PCR assays and the controls used are explained in detail in the Materials & Methods section.

2. In order to discuss more the association between HPV positivity and a subset of prostate cancers, an additional paragraph has been included in the Discussion (first paragraph).

3. Discretionary revisions:

   (i) The reason to analyze HPV in only 30 out of 48 hyperplasias is explained in the Materials & Methods section.

   (ii) We chose the order of HPV PCR assays (first TS-PCR, second consensus PCR) because it was in our hands more sensitive for the detection of HPV16.

   (iii) The doublet seen in Figure 1 is explained in the legend.

Response to reviewer Erik Wilander
The objections raised by Dr. Wilander have also been addressed by the other two reviewers and have been answered in our responses to their comments.

We hope that the revised manuscript is now acceptable for publication in BMC Urology.

Yours sincerely,

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