Author's response to reviews

Title: Testing of human papillomavirus in lung cancer and non-tumor lung tissue

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Author's response to reviews: see over
August 2, 2012

Dr. Mahboobeh Safaeian
Associated Editor
BioMed Central

Re: MS: 4700188757290441

Dear Dr. Safaeian,

Thank you for your willingness to evaluate a revised version of our manuscript “Testing of human papillomavirus in lung cancer and non-tumor lung tissue”, which we hope you will find suitable for publication in *BMC Cancer*. Enclosed please find the revised manuscript with changes in red font and a point-by-point explanation of the modifications in response to the reviewers’ comments.

Sincerely,

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Reviewer #1

1. It would be useful to have more information about how the frozen tissue was collected. Were the tissues immediately snap frozen in LN at the time of removal? How long on average was the time from removal from the vasculature to snap freezing?
Response: Within 5-10 min of removal, the tissue was put in plastic tubes and then frozen at -80°C. The manuscript has been revised to add this information (see p. 3, lines 22-23)

2. A little more description of the “strict procedures...developed to avoid specimen contamination” might be useful for readers to wish to perform similar experiments in the future.
Response: We have already explained that we used a pre- and a post-PCR area to avoid possible contamination. Additional strict procedures included standard good laboratory practices (e.g., frequent changes of gloves, use of disposable plastics, frequent cleaning of the work areas). We think that it is unnecessary to detail these standard procedures in the text.

3. The findings that repeat testing with a new assay kit lot failed to confirm initial weakly positive results seem particularly useful. It may be worth noting that other studies reporting on HPV in controversial areas could to use this approach as an additional way to help assess their findings.
Response: We agree with the reviewer’s comment.

4. While I tend to agree that these results argue against any pathogenic role of HPV in lung cancer, it should be pointed out that this study was conducted in a Western population and the majority of cancer cases (81%) were smokers. It may therefore be prudent to add the qualification “in this population” to the end of the Conclusion at the end of the paper, just as the authors did in the Conclusions section of the Abstract.
Response: We modified the Conclusions section according to the reviewer’s request. (p. 6, lines 2-3)
Reviewer #2

(1) The quality of DNA isolated from the samples is not discussed. Please provide a table that details the DNA yield and purity (260/280 absorbance ratio) from each sample.

Response: The quality of DNAs was good (mean 260/280 absorbance ratio = 1.98; total amount of extracted DNA ~10 μg/sample). Accordingly, all samples were successfully amplified using the CFTR gene as a genomic DNA control: we have modified the text to emphasize this point (p. 5, lines 8-9). However, we do not think that it is necessary to add a table reporting the 260/280 absorbance ratios, since they are raw laboratory data.

(2) This study lacks proper biological positive controls; this is essential for reporting a negative result. Ideally this would be human tissue with a known HPV infection, but at the very least an HPV-infected cell line such as HeLa cells should be processed alongside the lung tissues.

Response: We have added a paragraph describing the positive and negative controls that were used (p. 5, lines 1-6). We have also added a new figure (Figure 1) showing the results of a positive control as well as of a negative sample.

(3) As a recommendation for an additional figure, it would be helpful to show the original DNA gels of the CFTR internal PCR control to verify the DNA quality from each sample and the success of the PCR.

Response: We have added a new figure (Figure 1) showing a control for PCR (amplification of an internal plasmid) and a control for DNA quality (amplification of genomic DNA).