Author's response to reviews

Title: Genomic profiling identifies common HPV-associated chromosomal alterations in squamous cell carcinomas of cervix and head and neck

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Author's response to reviews: see over
Dear Prof. Norton,

Thank you for the evaluation of our manuscript 9987108623556258 (Genomic profiling identifies common HPV-associated alterations in squamous cell carcinomas of cervix and head and neck) by Wilting and Smeets et al and for giving us the opportunity to submit a revised version.

We have changed our manuscript according to the useful comments of the reviewers and have now included pathway analyses to move beyond mere descriptive changes. Furthermore, we discuss the potential role of a number of genes in hrHPV-mediated carcinogenesis. Our comments to the reviewers’ remarks are included below, in which all amendments made to the manuscript are indicated as well.

Hopefully, the amendments made are sufficient to process the manuscript for publication in BMC Medical Genomics.

Sincerely yours,

Renske DM Steenbergen, Ph.D.
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**Reviewer's report 1; Reviewer:** Nallasivam Palanisamy

**Comment:** BAC array data set is not available, through the URL provided, for view until June 2009.

**Answer:** We apologize for this oversight and have now made all array data available through the URL provided.

**Comment:** In my opinion, the major weakness in this study is not including a detailed description of the aberrations identified in this study. The CGH aberrations are presented in the same resolution and format as the CGH community used to describe the CGH data obtained using metaphase chromosome based CGH analysis. Although the resolution limit of BAC array CGH is about 1Mb, copy number changes can be better resolved than conventional chromosome based CGH. Loss of 13q21.1-21.33 and gain of 20p12.1-q13.33 and all common aberrations should be presented in much more details to accurate genomic coordinates and the list of BAC clones and the genes included in these regions should be provided.

**Answer:** We agree with the reviewer that our way of presenting altered regions undervalues the resolution obtained by the method we used and have now indicated the exact start and end positions of the regions in Table 2 and 3 (representing the significantly different regions between hrHPV-positive and hrHPV-negative SCCs and CxSCCs and HPV-positive HNSCCs, respectively). In Supplementary Table 1 a list of all BAC clones and genes located within the hrHPV-specific alterations at chromosome 13q and 20q is provided. In this table we have limited ourselves to the smallest regions of overlap in the hrHPV-positive carcinomas, as is shown in the added Figure 4.

**Comment:** Although these two cancer types share some common aberrations, do they share the same genomic boundaries; if not a minimal common region (MCR) should be defined. A refined analysis will likely identify a potential target genomic region or genes affected by HPV infection which will be a significant improvement towards understanding the genetic basis of these two cancer types with HPV involvement.

**Answer:** We greatly appreciate the reviewer’s useful suggestion and have now determined minimal common regions, which we called smallest regions of overlap (SROs), for the hrHPV-specific alterations at chromosome 13q and 20q. In figure 3 the genomic boundaries of all alterations on chromosome 13q and 20q are shown for the hrHPV-positive carcinomas and the SRO is depicted at the bottom of the graph. All genes located within the SROs are listed in Supplementary Table 1.

**Comment:** All the aberrations presented in Figure 3 should be presented at its actual genomic resolution and copy number level.

**Answer:** Although we understand the reviewers request for more detailed information on the altered genomic regions in Figure 3, we feel that this would be a reiteration of the data shown in Table 2 and 3. As we meant to provide the reader with a simplified overview of our most important findings, we have not changed Figure 3.

With respect to the copy number levels, the CGHCall algorithm we used for calling of gains and losses determines dichotomized copy number levels (-1= loss; 0=normal; 1=gain; 2=amplification), making it impossible to include the actual copy number levels.
Comment: I would like to suggest to include an independent validation using FISH to assess the copy number levels for both losses and gains.
Answer: In our opinion it is not necessary to include independent validation by FISH in this study since the reliability of the platform we used has already been extensively validated in previous studies (Van den IJssel, Nucleic Acids Res 2005; Van de Wiel, Bioinformatics 2007). In addition we have already shown FISH validation for the gain at chromosome 3q and MLPA validation for the gain on 20q in a subset of the cervical carcinomas also included in this study (Wilting et al, J Pathol 2006).

Reviewer's report 2; Reviewer: William Lockwood

Comment: The authors use array CGH data to define regions of copy number alteration specific to HPV-positive head and neck and cervical cancers. The approaches used and statistical methods employed are very sound and yield interesting results. However, it is disappointing that the authors do not interpret or discuss their findings to further insights into the mechanism of HPV mediated tumorigenesis. In its current state, the article solely lists regions of alteration common or specific to each group. The authors should take this information one step further and identify the specific genes and pathways associated with tumorigenesis in each group, particularly HPV positive cancers. For example, in the conclusions, the authors state that 32% of the genes found by Pyeon et al to be differentially expressed between HPV-positive and negative cancers were located within the regions identified in this study. It is unclear why the authors did not investigate these genes and their functions further. Are any of the genes known to interact with E6 and E7? What pathways are these genes involved in? How would this impact the treatment of HPV-positive vs HPV-negative cancers. Questions of this nature need to be addressed in order to relate the findings to cancer biology.
Answer: We appreciate the useful suggestions by the reviewer and have now performed pathway analysis on the genes located within the altered chromosomal regions. Details of the pathway analysis performed are included in the Methods section (page 7, lines 5-10). Results from this analysis are included in a separate paragraph in the Results section of our manuscript (page 9, line 25 and page 10, lines 1-19). Further interpretation of these results has been included in the Discussion section (page 12, lines 20-25 and page 13, lines 1-17).
With respect to the study by Pyeon et al, we believe that the relatively high percentage of overlap strengthens our findings. It should be noted however that only one gene (SYCP2) found by Pyeon was located within a hrHPV-specific chromosomal alteration. To the best of our knowledge none of the genes are known to directly interact with E6 or E7, which we have now indicated in the Discussion section (page 12, lines 16-17). To further investigate the potential functions of the overlapping genes, pathway analysis was performed on the “Pyeon” genes showing differential expression concordant to the chromosomal alterations we found. Results from this analysis are included in the Discussion section (page 12, lines 17-19). In general, cell cycle/proliferation appears to be particularly important for hrHPV+ tumours, which may be a reflection of the continuous E7-regulated E2F1 activation and is accompanied by changes in overall cellular maintenance systems.