Author’s response to reviews

Title: Exome sequencing characterizes the somatic mutation spectrum of early serrated lesions in a patient with serrated polyposis syndrome (SPS)

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Author’s response to reviews:
Dear Professor Lubinski,

Thank you very much for considering our manuscript “Exome sequencing characterizes the somatic mutation spectrum of early serrated lesions in a patient with serrated polyposis syndrome (SPS)” by Horpaopan et al. for publication. Please find attached the revised version (HCCP-D-17-00028 R1). We followed the comments and recommendations of the reviewers as described in detail in the revision protocol below and checked again that the manuscript follows the HCCP style and requirements.

Comments to the Reviewers

We thank the referees for their careful review and the constructive remarks which helped us to improve the manuscript. We followed the suggestions and recommendations as described below and give point-by-point descriptions on how we dealt with the comments. The changes in the text were underlined and marked in yellow.

REVIEWER # 1 wrote:

1.

Authors have a unique material and the postulate that the epigenetic changes may be important factor in serrated polyps development. My question is why they did not check it.

1. our comment

The aim of the present study was the systematic and exome-wide identification of point mutations in protein-coding genes as novel approach. Actually, exome sequencing data can cover
only a certain subset of alterations that might be present and contribute to tumorigenesis. Besides of epigenetic changes, mutations outside the coding regions (deep intronic regions, intergenic regions, promoters) and structural variants are missed. However, a comprehensive molecular approach covering all levels of possible alterations was beyond the scope of this study. We agree with the reviewer, that epigenetic changes are likely to contribute to the formation and progression of serrated polyps and thus, methylation analyses can further help to unravel etiologic factors of serrated tumorigenesis. Given the extent of previous studies regarding target methylation analyses in the field, novel and meaningful results can be expected in particular using a genomewide approach. We consider to extend the present study to capture genomwide methylation changes and structural alterations but since genomwide methylation pattern have to be compared with sufficient numbers of appropriate normal tissue and are complex and difficult to analyse and interprete, such an endeavour would be part of a separate study.

REVIEWER #2 wrote:

1.

The results, as presented, suggest that at the least the hyperplastic polyps in the descending colon with no BRAF or KRAS mutation do not have any other somatic mutations as part of early tumorigenesis, thus are the authors suggesting that the early changes are all epigenetic? Could there have been over-filtering of variants? The somatic mutation identified in the 7 SSA/Ps with BRAF in the Sakai et al study, mentioned in the discussion of this paper, were these variants (and other key serrated pathway genes like RNF43) checked for regions of low coverage or poor sequence mapping as potential reasons for not observing them in the current study?

1. our comment

Indeed, no KRAS or BRAF mutations were identified in polyps in the descending colon although the relevant genomic region was well covered in all polyps. That does not necessarily mean that only early epigentic changes are responsible for polyp formation or that the filtering was too stringent since the analysis of exome data cannot identify all relevant types of driver mutations. In particular, mutations outside the coding regions and adjacent intronic areas (deep intronic regions, intergenic regions, promoters) and structural variants are missed.
The variants in the candidate genes were filtered beforehand under less stringent criteria (compare page 8: Prior to further filtering and validation steps of the exome sequencing data …) so that overfiltering is unlikely to be the reason for the negative results.

We agree with the reviewer that low coverage and poor mapping might be critical points. To exclude false negative results for the published candidate genes, we now again carefully checked the regions of the 123 Sakai candidate genes, RNF43, and the other candidates and could exclude low coverage or poor mapping as reason for the negative results. The negative result might argue in favour of considerable genetic heterogeneity of somatic mutations in serrated polyps. This would also be in line with the findings of Sakai et al. since they identified somatic variants in a small subset of the 123 candidate genes only and those somatic variants as a second somatic mutation beside of BRAF or KRAS were found in only around one third of polyps. In addition, our negative results might be related to the smaller size of the polyps since in early polyps less somatic events are expected.

We now added this information in the manuscript. Please see page 8, Results, second paragraph; Discussion, page 10, last paragraph; and Discussion, page 12, first and second paragraph.