Reviewer's report

**Title:** Decreased expression of 17beta-hydroxysteroid dehydrogenase type 1 is associated with DNA hypermethylation in colorectal cancer located in the proximal colon

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**Reviewer:** Jarom Heijmans

Reviewer's report:

the study the authors present in their work shows that the gene 17β-hydroxysteroid dehydrogenase type 1 short transcript is more expressed in proximal colon cancer than in distal cancers. the authors show that this expression may be due to promoter CpG methylation differences. furthermore, this methylation may influence the turnover of E1 into E2 and thus change the outcome of cancer.

authors show convincingly that the promoter of HSD17B1 is methylated in cancer, and that this can be reverted by treatment of 5-dAzaC. the result of this methylation in terms if gene expression in patients and biological function however is less convincing to me.

major concerns:

authors show that in proximal cancers compared to healthy tissue the expression of HSD17B1 is downregulated. this counts only for the short and not the long transcript. if this is the result of methylation, would you not expect the gene itself to be silenced and not splice variants to be different. additionally, in figure 4, there seems to be no difference in long or short isoform mRNA expression after demethylation.

my advice is to depict the gene expression as expression of total HSD17B1 mRNA.

furthermore, in the results section on the expression of HSD17B1, a number of groups are described (female prox cancer, female prox healthy, female dist cancer, female dist healthy etc.) In the case of so many subgroups, a 2-way ANOVA analysis for significance is a more appropriate test to perform on all samples together.

minor concerns:

-authors state that important studies have been performed to assess the use of E2 in the treatement of colon cancer. however the largest study performed to assess this is a study done by the womens health initiative. where a large cohort of postmenopausal women are treated with placebo or estrogens. no decrease, possibly a slight increase in colorectal cancer was observed in women treated with E2. this study is not cited, but is of crucial importance for the objective of the
- the immunohistochemical analysis that are performed in figure 2 should depict healthy as well as cancerous tissue of proximal, distal and rectal colons. the healthy tissue seems at least dysplastic to me, it is advisory to discuss these pictures with a pathologist to evaluate his opinion about this. in addition, the staining for HSD17B1 seems to be predominantly in the vacuoles of goblet cells, i would think that staining localized there might be the result of unspecific antibody binding.

- immunoblots that are performed of tissue of cancerous or healthy material (figure 1c) seem to be performed or exposed at different time points. thereby a difference in HSD17B1 expression may seem obvious, however doing the westerblot in one run (or alternating cancerous and healthy material of one patient) may reduce this problem

in addition to these concerns, i think the study is well written, but a number of little errors concerning the language could be improved i give two examples of these errors:

sentence 272: we observed slightly DNA demethylation
this should be slight DNA methylation

sentence 303 Moreover, the cohort’s studies indicated that
this should be the cohort studies indicated that

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests