Reviewer's report

Title: Aberrant methylation of NPY, PENK, and WIF1 as a promising marker for blood-based diagnosis of colorectal cancer

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Reviewer: Sergia Velho

Reviewer's report:

In this paper, the authors describe three new methylated markers that can be used in the detection of colorectal cancer. In addition, they propose a methodology to study the methylation status of the genes in tissue samples and serum. The identification of colorectal cancer markers in blood samples is an important step for the development of minimally invasive, cost effective methods that can be applied in the early detection and prevention of colorectal cancer. In my opinion, the data reported by the authors is interesting, the question is well defined and the methodology is appropriate. The title, and the abstract reflect the results. However, I am unable to decide on acceptance before the authors address the following aspects:

Major compulsory revisions:

1. The authors should determine the CpG island methylation phenotype (CIMP) status in the samples analyzed in order to show that methylation of the proposed markers is a common event in colorectal cancer, in general, and not dependent on the presence of CIMP.

2. In the first section of the results (“Selection of candidate biomarkers by DNA methylation-array”) a table or figure showing the results and the genes studied is needed.

3. In the abstract, the authors conclude that: “… effective noninvasive test in preselecting symptomatic patients for colonoscopy and potentially in the mass screening of colorectal cancer prior to the colonoscopy.” I don’t agree with the conclusion that their assay can be used to select symptomatic patients for colonoscopy because shouldn’t symptomatic patients be always screened by colonoscopy in order to evaluate the reason (cancerous or noncancerous) of their symptoms independently of a negative result (lack of methylated markers)? I agree that, when validated, methods like the one described by the authors can be useful as a noninvasive, cost effective screening tool for the selection of asymptomatic cancer patients for colonoscopy but I am not sure of its legitimacy to determine if a symptomatic patient undergoes colonoscopy or not.

In my opinion, the authors should rephrase the sentence or explain in more detail their point of view.

Minor essential revisions:

4. A better description of how the genes were selected is important (some of this
information is described in the methods sections – Description of the clinical study, but, in my opinion, it would help to better understand the results if it was included in the results). In addition, it was a bit puzzling to me whether WIF was included in the study because it was found methylated in the array or if it was because WIF is known to be methylated in colorectal cancer and this way could be a potential marker in combination with PENK and NPY. I assume it came from the arrays based on what the authors wrote in other parts of the manuscript but I would like to see it better described.

5. Related with comment number 3, the authors state that their assay is also relevant to detect non colon cancers, so, a positive result does not necessarily means that the patient has colorectal cancer but it might have cancer somewhere else? I would like the authors to comment a bit more on this and what would be their suggestions if their assay was used in the clinics.

Discretionary revision:
1. In the results section – QM-MSP test in the sera for testing other cancers, in the last sentence the authors repeat the word sensitivity (“… sensitivity of 42% for a sensitivity of 80%). Do they mean sensitivity of 42% for a specificity of 80%?
2. What results did the authors get with stool samples? Would it be feasible perform the assay in stools instead of blood samples?

Level of interest: An article of importance in its field

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

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests