Reviewer’s report

Title: Expression levels and promoter methylation of mismatch repair genes in sporadic colorectal cancer in the Czech Republic

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Reviewer: Tatyana Vlaykova

Reviewer’s report:

Reviewer’s comments:

The manuscript entitled: “Expression levels and promoter methylation of mismatch repair genes in sporadic colorectal cancer in the Czech Republic” aims to investigate the mRNA expression profile and epigenetic alterations of MMR genes in tumors and adjacent mucosal tissue of 53 CRC patients with sporadic colorectal cancer from the Czech Republic. The manuscript is conducted with the aim to explore the interconnection between the expression and epigenetic alterations of MMR genes, which are involved in some proportion of sporadic CRC.

CRC as a focus of the current study is well argumented with the very high incidence of that neoplasia in the Czech Republic. The study is well designed and carried out. The methods are appropriate and reliable for obtaining trustworthy results. They are described clearly and in sufficient details, with the exception of the method for analyzing MSI status. The statistical analyses are also appropriate. The introduction, discussion and conclusions are adequately supported by literature overview, and obtained data. The results are interesting and may give a light on the process of carcinogenesis of colonic and rectal cancers.

However the work and the manuscript have several limitations and imperfection that should be considered and corrected. The most important one is the relatively small number of CRC patients studied (n=53), which requires caution in the discussion of the results and especially in doing conclusions.

I consider that the manuscript is worth to be published after some corrections.

Major Compulsory Revisions:

1. I do not agree with the term “healthy tissue” which is usually used in the text of the manuscript to define the adjacent mucosal tissue. These parts of the colon/rectum are apparently not affected by the tumor process, but could not be considered as “healthy tissue”. In this respect, I suggest the used terms “healthy mucosa” or “healthy tissue” to be replaced in the whole manuscript (including tables and figures) with the more correct terms “adjacent mucosal tissue” or “adjacent non-affected mucosal tissue” or “adjacent non-affected mucosa” or “non-affected mucosa” etc.
2. The methods for analyzing the MSI status should be given in more details: temperature profile for the multiplex PCR, the primers of microsatellite markers, or at least should be cited papers with those details.

Similarly, some more details for Methylation-specific PCR are required – at least the annealing temperatures should be given in table S1a and the method for distinguishing of the PCR products of the amplifications with primers for methylated and unmethylated templates (agarose or PAAG electrophoreses, or some other way).

3. It was stated that 2 reference genes were applied as reference genes ACTB and 18S rRNA and “Data were normalized to reference genes,...” How did you performed the process of normalization to the 2 reference genes – how did you decide which of the studied genes to be normalized to ACTB and which of the MMR genes to 18S rRNA? Isn’t it more correct all of the studied genes to be normalized to one common reference gene? How can you explain the application of 2 reference genes?

4. Due to the fact that MSI is more often detected in carcinomas with proximal than distal colon localization, I suggest this characteristics of the colon cancers to be included in Table 1 and used further in the analyses.

5. Figure 1 and 2 are not clearly presented: there is no information what the bars presents – are they the mean values ± SD or medians with the 25-75 percentiles. I suppose that the bars present mean values, however as it was stated in the statistical section – the data for the expression of the genes had non-normal distribution – thus the data should be graphically presented with box and whiskers plots.

The information about the way of presenting the results in the figures should be stated in the text to the figures.

6. I have concerns about the results in Table S3b: the data are presented as mean values, but not as medians and 25-75 percentiles as it was performed in the other tables and as it should be. In addition the values of the “fold change” according to the tables seem to be calculated by the ration of the obtained mean values for “healthy tissue” and “colon cancer”. This is not a correct way. The fold change should be calculated for each particular patient and then found out the mean (median) fold change, depending of distribution.

7. Photographs of the electrophoreses (if such have been used for determination) of methylation-specific PCRs for promoter hypermethylation of MLH1 should be presented as an additional figure.

Minor Essential Revisions

1. The title is adequate to the performed work, but the aim in the abstract and in the introduction part is not correctly presented: “characterizing genetic/epigenetic profiles” and “to investigate the molecular (epi)genetic profile of MMR genes”

In fact in the work there are epigenetic analyses, but no genetic analyses. Studies on the mRNA expression should not be considered as genetic analyses, but as gene expression profiling. In this respect, the aims of the work should be
reformulated more correctly.

2. I suppose that “comprehensive screening for mRNA expression profile of MMR genes” of the population of Czech Republic (page 4, paragraph 2), or any other population is not reasonable, but analyzing mRNA expression profile of MMR genes in patients with CRC has a great sense. In this respect this first sentence in page 4, paragraph 2 should be rewritten.

3. In paragraph 2 page 6 there are many non-common abbreviations such as, GE sample loading reagent), UNG, ACTB, which should be given with full names also (uracil-N glycoslyase, actin beta) if possible.

4. Is there association of MSI with proximal/distal colon localization? (page 8, paragraph 3)

5. Correcting the format and abbreviation of the name of the journals in references 1, 14, 17, 19, 23 (omit - : an official journal of the American Association for Cancer Research), 24, 26, 27, 28, 29, 30, 31, 33, 34, 37 ( omit-official journal of the American Society of Clinical Oncology), 37.

All names of the journals should be in abbreviations following the Index Medicus/MEDLINE style.

5. Page 3, line 11 and page 14 - List of abbreviations – CpG means “Cytosine phosphate Guanine” but not “Cytosine polyguanine”. This term should be corrected.

Discretionary Revisions

1. The source of TNM system (Union for International Cancer Control (UICC) or American Joint Committee on Cancer (AJCC)) should be mentioned (page 5, paragraph 2-end).

Are the TNM stages presented in Table 1 for the patients are given by clinical examination or by pathological examination – this should be presented in table 1 (cTNM or pTNM system).

2. Page 11. “In our study, the presence of promoter methylation of MLH1 gene was also not related to the mRNA levels”. How could you explain this observation?

3. Page 9, line 12 – “demographic” instead “biological” factors

4. Page 10, line 6 – the p-value from the analyses of MSI and promoter methylation analyses of MLH1 should be presented.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

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