Author's response to reviews

Title: Analysis of MLH1 and MGMT expression and promoter methylation on genomic instability in patients with thyroid carcinoma

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Author's response to reviews: see over
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Dear Editor,

Please find enclosed the revised version of the Manuscript “ANALYSIS OF MLH1 AND MGMT EXPRESSION AND PROMOTER METHYLATION ON GENOMIC INSTABILITY IN PATIENTS WITH THYROID CARCINOMA” by Santos et al. Again, we thank you and the reviewers for the thoughtful and helpful review of our manuscript which certainly improved the manuscript. We believe we have addressed all concerns raised by the reviewer.

We hope you will find the revised version of our manuscript acceptable for publication in *BMC Cancer* in the present format.

Thank you very much for your attention.

Sincerely yours

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Response to Reviewer 1:

Major compulsory revisions

1. "The change of the incidence rate is not comprehensible if referred to the global situation or to a specific local setting (first paragraph of the Introduction)."

I agree with the Reviewer, and it is corrected in the new version of the manuscript.

2. "It should be identified the location where their cohort was followed-up in the Introduction when the Authors describe their previous studies (third paragraph of the Introduction)."

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

3. "The assumptions behind the computations of the sample size should be described in the Methods; moreover, it is helpful to explain why they used that sample size (i.e., no. 96) and that proportional distribution of tumors (first paragraph of the Methods)"

I agree with the Reviewer, and the explanation was included in the introduction.

"Papillary Thyroid Carcinoma (PTC) is by far the most common subtype, representing approximately 80% of cases. Follicular Thyroid Carcinoma (FTC) is the second most prevalent subtype, accounting for 10-15% of thyroid cancers”.

4. “They should explain in the Statistical analysis section how they describe the quantitative variables in terms of central tendency and variability indicators (Statistical analysis of the Methods)?"

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

5. "I suggest to change the word association in the text with relationship"

It was changed in the text.
6. “It is not understandable how the Authors showed the quantitative data; it seems that they used means and SDs; I would propose to use medians and IQRs for non-parametric variables (Results)”

It was changed in the new version of the manuscript.

7. “I would recommend to include the 95% CIs for the prevalent data (Results)”

It was included.

8. "Tables (no. 3 and 4) should display precise p-values for all the statistical significant and not significant comparisons they did. Moreover, more p-values should be included in the text when the Authors illustrate the statistical comparisons (Tables and Results)"

I agree with the Reviewer concern, and it is included in the new version of the manuscript.

**Response to Reviewer 2:**

**Major Concerns:**

1. “In my opinion, the study is not clear with respect to research hypothesis because the Authors first speak about the role of methylation and/or MSI in tumourigenesis of thyroid carcinoma and, later, about MLH1 and MGMT expression and methylation on MSI in patients with thyroid carcinoma harboring several mutations. These two sentences are counteracting according to me. Furthermore, if the attention is only on patient with cancer, why does the total number of samples encompass also normal tissue? Moreover, it is not possible to investigate tumourigenesis with such a type of study design (which is cross-sectional). Finally, at the end of introduction, the Authors stated that they focused on thyroid carcinoma harboring BRAF V600E, RET/PTC and IDH1 but they addressed not only

   Regarding the research hypothesis, I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

2. “The outcome measures are not specified; indeed also statistical analysis is difficult to be judged”
I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

3. “Since the lack of a hypothesis, it is quite difficult to go trough results because it is not clear the order of analyses (it seems that malignant lesions were compared to benign ones but it is not stated anywhere). Furthermore, were all analyses performed after stratification for cancer type?”

I agree with the Reviewer concern, and it is clarified in the new version of the manuscript.

4. “The sampling and the enrollment are not specified at all with respect to process, setting, time and representativeness”

I agree with the Reviewer concern, and it is clarified in the new version of the manuscript.

5. “Authors seem to have performed multiple comparisons when dealing with differences between PTC, FCT and benign lesions. Indeed, a correction for multiple tests should be adopted”.

It was adopted and included in the manuscript.

6. “Tables are confusing and numbers are inconsistent (i.e. the number of MSI-H, MSI-L and MSS samples do not sum up to 70 among PTC. Furthermore, I would expect to see the same denominator in the rows dealing with BRAF 600E and IDH1 in Table 3 because I would like to know the number of samples harboring the mutation on the total number of methylated and non methylated MLH1 and MGMT. In Table 4, the Authors described 50 MSI but reported 20 MSI-L and 39 MSI-H for a total of 59 samples - not 50! - Again, in Table 4, it is not clear at all what “Yes” and “No” were referred to. In Table 5, I do not see the information about MSI.

I agree with the Reviewer concern, and the Tables were corrected in the new version of the manuscript.

7. “Some statements in the discussion are not justified by results (i.e. “A significant relationship between MSI status and histological subtypes is demonstrated”: the Authors did not compare different histological subtypes; if they did, this was not specified in methods and was not dealt with appropriate statistical tests. Another statement not supported by data is the following: “The frequency of these markers is
significantly different compared with the other markers”: the Authors did not apply any statistical method to address it).”

I agree with the Reviewer concern, and it is removed new version of the manuscript.

8. “Limits of the study were not discussed at all and I am concerned about selection bias”

I agree with the Reviewer concern, and it is included in the new version of the manuscript.

**Minor Concerns**

1. "The title does not spread the aim and the content of the paper”.

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

2. "In the abstract Authors spoke about patients but actually they should speak about samples.”

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

3. “In the Introduction, Authors told that thyroid cancer is the most frequent endocrine cancer: this is true but should be referenced”

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

4. "In the Introduction, rationale of the study is completely lacking”.

I agree with the Reviewer concern, and it is corrected in the new version of the manuscript.

5. “In Results, the Authors presented some number but did not specify if they were mean and standard deviation. This should also be said in methods.”

I agree with the Reviewer, and it is corrected in the new version of the manuscript.

6. “The first part of Results concerning Microsatellite Instability should be better referred as method”
I agree with the Reviewer, and it is corrected in the new version of the manuscript.