Reviewer's report

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

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Reviewer: Chiara de Waure

Reviewer's report:

The paper “Effects of MLH1 and MGMT expression and promoter methylation on genomic instability in patients with thyroid carcinoma” should deal with the role of methylation and MSI in thyroid cancer but presents several criticisms and inconsistencies which make it unsuitable for publication.

Please find below major and minor concerns.

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.

2. The outcome measures are not specified; indeed also statistical analysis is difficult to be judged.

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?

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

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.

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.

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).

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

Minor Concerns

9. The title does not spread the aim and the content of the paper.

10. In the abstract Authors spoke about patients but actually they should speak about samples.

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

12. In the Introduction, rationale of the study is completely lacking.

13. 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.

14. The first part of Results concerning Microsatellite Instability should be better referred as method.

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests