Reviewer's report

Title: Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data

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Reviewer: ALFONSO DUENAS-GONZALEZ

Reviewer's report:

General
This work by Yang and colleagues investigated the methylation status at the promoter of 34 genes in a group of gynecological malignancies by MSP analysis. This is an interesting piece of work that could be much improved by considering the following observations

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The background in the abstract just provides the objective of the study and repeat what is stated in the method section. A short paragraph on the methylation field is required. In this sense, the methods section should describe the patient population studied, the techniques employed and the statistical analysis used for study correlations and survival.

Background.
The basis on which authors decided to study these three gynecological cancers can not be justified on the “correlation in the embryonic development of ovarian epithelium, endometrium and cervix”. The etiology, and molecular physiopathology are quite different for these tumors, for instances, cervical cancer has a clear viral etiology; the endometrial cancer is clearly a hormone-dependent neoplasm and ovarian has other potential causes. The fact that authors wanted to compare the role of CpG methylation for the development and progression of these tumors on the basis that tumors share a similar embryonic development is not therefore supported. The last sentence in the Background section “Potential epigenetic markers for diagnosis, prognosis and prediction of treatment outcome in the three cancers were also identified” is to strong. It was tried for cervical cancers but not for endometrium and ovarian.

Methods.
Authors should clearly specify whether the “normal tissue” was from blood in all cases or included “adjacent healthy tissues”. A description of the PCR controls should be added.

Results.
It is intriguing that FHIT was found methylated in “all” normal DNAs at least in the representative cases showed in figure 1. Authors should point out this finding in the discussion. Authors report that 4, 4 and 5 genes were aberrantly methylated in cervix, endometrium and ovarian cancer and that “for other loci aberrant methylation was not indicated in any of the three cancers because methylated alleles were present in both tumoral and normal DNA or methylated allele was not detected in both. This should be presented in detail as it is not clear at least for this reviewer. This reviewer also wonders why if authors found genes specific for each of these tumors, they chose to study other such as p16, APC and PTEN in cervical cancer and the same for the other tumors.
The analysis of the methylation and therapeutic outcome an prognosis in cervical cancer should be better described. Because cervical cancer was studied more in deep a separate table should be added for cervical cancer, and of course, treatments must be described more in detail and also included in the multivariate analysis. Is not clear for this reviewer what means “clear information of recurrence”. To include only 100 patients having this tag could be a source of bias. It is also confusing that for the OS and DFS survival, 116 patients were analyzed, this means that 16 patients with “unclear” information of recurrence were also analyzed?

Regarding the evaluation of response prediction, which were the pathological findings to consider a tumor radioresistant or radiosensitive? Was the biopsy taken after external radiation or after brachytherapy if done? Which was the treatment delivered?

Authors should also better describe the endometrium and ovarian cancer patients and the analysis for supporting the lack of association between methylation and recurrent diseases in those patients.

Discussion

The findings of the methylation at the endometrium and ovarian cancers should be discussed with other published results on the methylation at these genes in these tumors. The same apply for the findings in cervical cancer. There are at least four studies on cervical cancer analyzing methylation at these genes that should be discussed. The discussion on the meaning of DAPK gene for predicting response and prognosis should remain only if authors clarifies the issues raised on the “radioresistance” and on the recurrence assessment, also should also discuss on the marginal significance on prognosis (DFS) (p=0.041) in the multivariate analysis. On this regard, it could be useful to have an expert statistician reviewing the manuscript.

Conclusion.

As stated before, the last sentence of the conclusion “This epigenetic event might be a potential molecular marker in diagnosis, prognosis and treatment of gynecological cancers” is essentially correct, however, this can not be assumed from this work. I would better suggest something like “more studies are needed to define its potential role.......”

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: Yes

Declaration of competing interests:

I declare that I have no competing interests