Reviewer's report

Title: Cross platform analysis of methylation, miRNA and stem cell gene expression data in pediatric germ cell tumors highlights characteristic differences by tumor histology

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Reviewer: Rui Henrique

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In this study, Pointer and co-workers analysed DNA methylation, miRNA expression and stem cell gene expression across pediatric germ cell tumor types. They observed differences among germ cell tumor types, with yolk sac tumors demonstrating a quite distinct (epi)genomic profile.

The methodology seems globally sound (but see below) and the results are interesting, confirming some previous published findings from other research teams, but also providing novel information that might be of clinical importance for the therapeutic management of germ cell tumors.

Major compulsory revisions:

1. It is not clear what do the authors consider "pediatric" germ cell tumors. Using age as a parameter for discrimination (pediatric age ≤ 18 yrs), then some cases fall outside the definition. In this context it is odd that a dysgerminoma from a 45 year old woman was included. The authors state that it was due to its similarity in (epi)genomic profile with adolescent dysgerminoma. However, this could bias the results. Why not to include other dysgerminomas that may have a different profile? This must be fully clarified.

Notwithstanding, "pediatric" germ cell tumors might also be defined (as they are pathologically) as those arising before puberty. This is probably the most important definition, because using this definition, "pediatric" GSTs are unrelated to intratubular germ cell neoplasia unclassified (IGCNU) and consist essentially of yolk sac tumors and teratomas. Owing to the association of post-pubertal GST with IGCNU (particularly seminoma) it seems obvious that the (epi)genetic landscape would be different.

Thus, the discussion of the results should be made in light of these concepts, as they provide further insight into the pathogenesis of GST, especially the "pediatric"/pre-pubertal types. Interestingly, concerning miRNA expression analysis, the authors compared tumors diagnosed prior to age 10 years vs. those diagnosed at or after 10 years of age (page 11, line 9). What is the underlying rationale for this subdivision?

2. There is some confusion regarding the classification of the GSTs included in this study. In the Results section and Table 1, there are 4 cases of "mixed histology". However, in Figure 1, this category is not represented. On the other
hand, in Figure 1, immature and mature teratomas are discriminated, but this is not evident in Table 1 or the text, nor in Figure 2. These issues require full clarification.

3. It is stated that 7 normal adjacent tissue samples were also analysed. Considering the issues raised in Comment 1, the nature of these samples must be explained and characterized. Were these from testicular tissue? Were they from "pediatric"/pre-pubertal cases (meaning that IGCNU is not present in the adjacent tissue)?

4. Please provide an explanation for choosing the 0.75 threshold (page 9, line 14).

5. The Discussion is too long and should be shortened.

Minor discretionary revisions
1. Page 4, line 12: the term "intratubular germ cell neoplasia" is more often used that "carcinoma in situ". Please replace or include as equivalent terms.

Level of interest: An article of importance in its field

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