Reviewer's report

Title: Predominance of Triple Wild-Type and IGF2R Mutations in Mucosal Melanomas

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Reviewer: Zofia Helias-Rodzewicz

Reviewer's report:

Title: Predominance of triple Wild-Type and IGF2 Mutations in Mucosal Melanomas.

Yuuki Iida and colleagues present the molecular study of Mucosal Melanomas (MM) by next-generation sequencing method in order to investigate its mutation profile. MMs are rare and aggressive non-cutaneous melanomas, non-related to sun exposure. They have already been studied together with other melanoma subtypes; however, studies in large MM groups are less frequent. Thus, this article adds new knowledge of MMs.

1) Page 5: DNA extraction

No information is given about the percentage of tumour cells into the extraction area. Even if most melanomas are cancers with high % of cancer cells, a large proportion of samples may have <80% of them. Furthermore, melanoma is a highly aggressive and heterogeneous tumour. The choice of tumour cell rich areas could give more relevant results and identify mutations with low frequency.

2) Page 6: variant calling and data analysis

Variants with "mt allele frequency >10%, and coverage >20x were used for further analysis". 20x is rather a mild criteria for the selection of new variants. NGS could miss hot spot mutations even in frozen tissue with coverage as low as x50. Higher coverage should be expected in searching for new variants in less known cancers.

3) Page 8: Results

The authors state that the C>T substitutions were significantly less in MM (57.8%) than CM (64.8%) supporting less involvement of UV exposure in MM. This statement is rather confusing because we expect that the UV exposure has no effect on MM development. Some MM specimens present signatures for tobacco exposure, and the authors conclude that smoking is a potential pathogenic factor in MM. Have the authors the information about the smoking history
for these patients to confirm this suggestion? Other explanations are possible for the signature of tobacco exposure and the genital and anorectal localisation of MM cancers?

4) DCC antibody should be tested to strengthen its role as a prognostic marker. How DCC mt modify protein expression and its role in apoptosis?

5) Figue 1B abscissa legend is illegible.

6) Recently published NGS analyses in mucosal melanomas are not included and listed in the article.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
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I am able to assess the statistics

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