Reviewer’s report

Title: Whole exome sequencing of benign pulmonary metastasizing leiomyoma reveals mutation in the BMP8B gene

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Reviewer: Netta Mäkinen

Reviewer’s report:

Here, the authors have performed whole exome sequencing on a pulmonary metastasis and peripheral blood sample from a patient diagnosed with benign metastasizing leiomyoma (BML) to examine somatic mutational landscape of pulmonary BML. They identified a missense mutation in BMP8B gene, suggesting the gene to have a facilitating role in the metastasizing of BML. Also results from X-chromosome inactivation assay proposed monoclonal origin of the pulmonary BML.

Since this manuscript is based on data from only one BML patient, a case report could be a better-suited format for the paper instead of a research article.

Major concerns:

1. Because the manuscript is based on data from only one pulmonary BML, it makes it rather difficult to draw conclusions from the data.

   - Without validating the BMP8B mutation further (more samples, presence of the mutation on cDNA level, etc.), it is difficult to rule out the possibility that this mutation is just a passenger change.

   - Could it be just a coincidence that both the pulmonary metastasis and uterine leiomyoma showed non-random X-chromosome inactivation with the same allele being inactivated?

2. Filtering criteria for the exome sequencing data:

   - Why were the splice variants not taken into account in the analysis? (exon-intron boundaries are usually well-covered in the exome data)

   - SIFT and Polyphen2 do not give predictions to indels, thus did the authors exclude all indels from the analysis? Could an additional in silico prediction program be used for indels?

   - Why were the heterozygous mutations required to harbor similar depths between the alleles? How did this affect the filtering of the variants? Were some variants excluded from the analysis based on this criterion?
-Due to the criteria (4), is there a possibility that the authors are missing potential novel candidate mutations?

Minor comments:

Introduction:

-line 69: Since chromosomal aberrations are also mutations, it would be recommended to use another term than 'mutational landscape' in this case.

Methods:

-line 79: addition of 'uterine' before leiomyoma

-line 84: Did the authors mean 'smooth muscle cells' instead of 'leiomyoma cells'?

-line 89: Was the quality score of extracted DNA the same (0.8) for each FFPE sample?

-line 93: In the beginning of the Sequencing paragraph, it would be preferable to describe, which samples entered exome sequencing.

Results:

-lines 178-179: How did the authors define the absence of CNVs? Did they use any specific software designed for exome data or manually compare the coverages between the metastasis and peripheral blood sample?

-Why was the endometrioma sample not part of the X-chromosome inactivation analysis?

Figures:

-Figure 2: Addition of the used magnification to the figure legend.

Ethics approval:

-The used informed consent form does not need to be approved by the ethics committee?

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.

Yes

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

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

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