Reviewer’s report

Title: Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology

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Reviewer: B. C. Ruetgen

Reviewer's report:

Comments to the manuscript "Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology" for "BMC Cancer" BCAN-D-18-00269:

Comments to the Subject Editor

The topic of the submitted article fits very well into the general scope of „BMC Cancer“.

The innovative aspect of the data is high, new established and well characterized cell lines are always of outstanding importance for the scientific community. Nevertheless the presented results are not described in sufficient detail and consistency (Material+Methods and Results). The manuscript is partly difficult to read and to follow. It is often not clear from which material the cell lines were established. Was it the primary material or was it the xenograft material? Which passage of what? This has to be defined precisely. The naming of the cell lines is often not consistent - eg Fig 9: Dog_1cell line and Ocr_OCMM1 and Ocr_OCMM1 passage1. Sometimes ocr instead of Ocr. This is confusing.

Another point is, why was the primary material injected in mice? And at which time point? Why were the cell lines not derived from the primary material without xenografting?

Some of the Additional figures are not referenced or mentioned in the text - this is another point to be clarified.

Some methods evaluated for characterization are not further strengthened in the results and vice versa.

Because of all these important open questions I recommend the manuscript "Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology" to be published in "BMC Cancer“ exclusively after MAJOR REVISION.
Some comments on the manuscript:

Introduction

Cell lines are always models for the species itself and also in comparative ways. The Introduction is at the beginning confusing. It would be recommended to delete the whole model introduction part and to more streamline the introduction towards the focus of the manuscript - melanoma cell line establishment and characterisation. It is proposed to start with:

Page 5 line 102: In humans,............

Material and Methods

"Tumor samples" and "Isolation and cell culture":

It is not 100% clear what was done when. The primary material was gained. Was it then immediately injected in the mice after digestion and short phase in cell culture and if not how many passages was it in the cell culture?

It is a bit confusion - are there 2 cell lines of each Dog? The one from the primary material and one from the xenograft? And what is meant with passage 1 and 3. Was this after gaining the tumor from the mouse? Is this then the cell line?

Another thing which has to be added is the information how long the cell line was continuously cultured? A cell line line can just be called a cell line if it was in continuous culture for minimum 9 month, correctly one year. In Table 1 there are only the passages mentioned and the doubling time. This should be explained in the text. As mentioned on page 8 line 185.... passaged several times..... is not sufficient.

As mentioned also in the general comment at the beginning, the cell lines should be named consistently, to avoid confusion. In Table 1 it says for dog 1: Ocr_OCMM1 and then Ocr_OCMM1 passage1, for dog 2 Ocr_OCMM2 primary cells and then Ocr_OCMM2. Please clarify.

"First Xenografts", "Inoculation of melanoma":

As mentioned before, what was exactly injected. It is said that the cells went in a T75, and here the primary cells were injected immediately. And if not which passage was injected?

Was primary material and "cell line" injected?

Immunohistochemistry:

Here it is said, collected tumor tissue (page 10 line 235). From the dog or the mouse? Or both?

Pharmacological characterization
MTS cytotoxicity assay –

Please give the information to the abbreviation MTS, and again which cells were used.

Western blot

Again page 12 line 266: which cells, please be precise

Results

Establishment and characterization of canine melanoma cell lines

Please also here describe how many passages more precise, which cells and when? - this should be streamlined through the whole manuscript.

Is ocr equivalent to Ocr (see page 14 line 336+337)?

Genomic characterization

Page 18 lines 417-428 - please explain this in more detail and this also has to be discussed, because these results cannot be found in Figure 9 as referred.

Discussion

At the beginning of the Discussion section it is mentioned that there are already existing cell lines for oral/gingival canine melanoma - maybe this could already be mentioned in the Introduction.

Figure 3: it would maybe better to write cell line instead of cell lines. Or how many cell lines were created from the 5 mice?

Figure 4B: please add the information where the Figure is mentioned in the text

Figure 5: in the text and also in the Figure legend mitoses are mentioned. Pictures with higher magnification would be helpful to see the mitoses.

Figure 8: please modify the Figure legend, neither in the text nor in the figure legend the control: HSC-70 is mentioned. This has to be documented. pMEK, MEK, pAKT, AKT is not mentioned in the figure legend, please complete the information.

Additional files 2, 3, 4, 5 are not mentioned in the manuscript. Please reference them.
Conclusive remarks

I recommend the manuscript "Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology" to be published in "BMC Cancer" exclusively after MAJOR REVISION.

**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.

Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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