Peer Review File

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Reviewer #1:

Comments:

This study is a very important review of currently available preclinical models of three tumors in ophthalmic oncology: conjunctival melanoma, uveal melanoma, and retinoblastoma, which provide a very interesting contribution to the scientific academy.

Specific comments and criticisms:

Comment 1:

1. Introduction

1) Line 86 – Please, provide the abbreviation of Uveal melanoma – "Uveal melanoma (UM) is the most common melanoma…."

Response 1 : Abbreviation was provided.

Changes in the text : Changed in line 91.

Comment 2:

2) Line 113-115 – Please provide an actualized reference for the affirmation: "Retinoblastomas are the most common tumors of the eye in childhood and occur in about 1 in 15,000-20,000 births", since the references in the final of the paragraph are outdated – references 59 and 60. These statistics are from all over the word or in the US or Europe? This point needs to be explained.

Response 2 : An actualized reference was provided for statitics in EU.

Changes in the text : Changed in line 119.

Comment 3:

3) Line 120-121 – Please review the statement that: "Classic chemotherapy is considered the standard of treatment, since novel targeted drugs have yet to be established (61-66)". It is important to mention that other treatments than chemotherapy like cryotherapy, plaque radiation, thermotherapy, external

beam radiation, or a combination of such treatments are been used to treat retinoblastoma. Please revise your statement in agreement with the data in the literature.

Response 3: We provided more sources for other treatment options and changed the statement.

Changes in the text : Changed in line 126.

Comment 4:

2. Methods

1) Line 130 – Way the authors did not use the electronic bibliographic database web of science, one of the most important databases?

Response 4: We also used Web of Science for literature in revision, so we updated our list of tools in Methods.

Changes in the text : Changed in line 186.

Comment 5:

3. Discussion

1) Line 172-174 – Please, provide references for the statement in this paragraph.

Response 5: References were provided for this statement.

Changes in the text : Changed in line 228.

Comment 6:

2) Line 259 - Patient-derived xenografts (PDX) was already mentioned and abbreviated in line 176, please use only the abbreviation PDX and standardization in all text.

Response 6: Term was changed to abbreviation.

Changes in the text : Changed in line 319.

Comment 7:

3) Line 299-302 – Please, provide references for the statement in this paragraph.

Response 7: References were provided for the statement and updated in bibliography

Changes in the text : Changed in line 364.

Comment 8:

4) Line 369 – Please correct the expression: xenograft.models delivered…

Response 8: The expression was corrected.

Changes in the text : Changed in line 429.

Reviewer #2:

Comments:

The manuscript summarized the literature of preclinical models in conjunctival melanoma, uveal melanoma, and retinoblastoma. The authors discussed the advantages and limitations of these models.

Detailed Comments

Comment 1:

1. Where is conjunctival melanoma arise from? Why challenging to access the cell line and establish a preclinical animal model? Does a 3D cell culture model use the established conjunctival melanoma cell line in vitro?

Response 1: While CM arises from melanocytes, CM can form from three different precursors: primary acquired melanosis (PAM) in 74% of cases, nevus in 7%, and 19% arise spontaneously de novo. It is challenging to access cell line, because CM is very rare cancer entity and primary tumor biopsies are difficult to establish in cell culture. The 3D cell culture model used CRMM1 and CRMM

Changes in the text : Changed in lines 70 and 241.

Comment 2:

2. The authors mentioned the several selected cell lines are available for xenografts animal model in each eye oncology category. Please make a list of cell lines. How are these cell lines applied to individual animal models?

Response 2: According to Cellosaurus, although there are only 7 cell lines described in the literature for CM, there are 104 for UM and 252 for retinoblastoma, so a detailed list is probably beyond the capacity of this journal publication.

Comment 3:

3. The authors described PDX models in uveal melanoma often utilize SCID mice. The model of PDX is not only SCID but also NSG. In addition, diffluent sites of tumor inoculation and success rate have been reported. Please include more detailed information in the section.

Response 3: We included more information on NGS mice and studies on different oculation sites and updated the reference list.

Changes in the text : Changed in line 307 and 322.

Comment 4 :

4. Briefly describe any experimental result is reported in the zebrafish model.

Response 4: We gave a more detailed description on zebrafish models in text

Changes in the text : Changed in line 338, 430.

Comment 5:

5. Suggest making subtitles of each preclinical model and put together in the same category of the animal model. E.g. after an explanation of PDX, the authors described zebrafish and chicken embryos, and then back to the description of engineered mouse models.

Response 5: Our first draft was organized by model type, but then it didn't quite meet the guidelines for Narrative Reviews, so we ended up describing it as a coherent text after all. If desired, we can change this back in consultation with the editorial team.

Changes in the text : Not changed yet.

Reviewer #3

Comments:

This manuscript delivers a concise review on three tumor types of the eye that is of direct relevance to a clinical audience. In my opinion some extra introduction (or some restructuring) is required to better situate the different animal models. At the moment there is too much of a 'summing up'.

Recommended additions/changes:

Comment 1:

1) In addition to what is written now, the introduction should also presen the reader with the different forms of animal models that are typically used to model cancer : autochtonous model (= GEMs), transplantation models (both allografts and xenografts) and briefly mention the key aspects (e.g. immune cell involvement and tumor microenvironmment) which are further documented in Table 1.
The results can then better refer to these models in a logical structured way (autochtonous GEMs > allografting > xenografting > non-mammalian models).

Response : A brief introduction was added to the manuscript, shortly explaining the different models.

Changes in the text : Changed in line 134.

Comment 2:

2) Methodology: the authors seem to have missed some relevant autochtonous GEMs in other species than the mouse. They may hence have to extend the keywords in their search (e.g. with specific animal species). For instance they missed a model for uveal melanoma in the fish Medaka (PMID: 19609310) and a relevant genetic model for retinoblastoma in Xenopus tropicalis (PMID: 27739525), the latter showing a nice parallel with the mouse concerning the redundancy of rb1 and p107/rbl1.

Response 2: We provided a more detailed description and added tstudies and updated the reference list. We discussed to include the Medaka model, but since it is primarily a cutaneous melanoma study describing the occurrence of uveal melanoma as well, we decided to not include the study. Due to the genetic difference in cutaneous and uveal melanoma, to our opinion it is not right to consider it a typical uveal melanoma study.

Changes in the text : Changes in line 164.

Minor remarks:

Comment 3:

1) Gene names have to be written in italics.

Response 3: All gene names were checked and corrected

Changes in the text : Checked every gene name.

Comment 4:

2) line 153: rabbits are no rodents (they are Lagomorphs)

Response 4: Expression was changed.

Changes in the text : Changed in line 209.

Comment 5:

3) line 372: GFP is a fluorescent protein, not a luminescent (luciferase is the latter)

Response 5: False expression was corrected.

Changes in the text : Changed in line 435.

Comment 6:

4) line 378: the Langenau lab has managed to keep zebrafish at 37 oC for transplantation studies with human tumor cells (PMID: 32826993)

Response 6: The information was updated and changed in text.

Changes in the text : Changed in line 445.

Comment 7:

5) line 380: the GEMs for retinoblastoma are knockout (or conditional KO). The term 'transgenic model' is more fit for an overexpression model. In any case the term genetically engineered model (GEM) covers both.

Response 7: The term was changed to “knockout”

Changes in the text : Changed in line 450.

Comment 8:

6) line 397: use correct gene nomenclature (e.g. Rb1 and not RB1 for the mouse gene (evidently in italics)) and include correct HUGO names (p107/Rbl1 and p130/Rbl2).

Response 8: Gene nomenclature was updated.

Changes in the text : All gene names were checked and updated.

Comment 9:

Table 1: replace 'Transgenic/induced models' by 'Genetically Engineered Models'

Response 9: The expression was replaced.

Changes in the text : Changed in Table 1.
