Reviewer's report

Title: A short-term in vivo model for giant cell tumor of bone

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Reviewer: Sofia Avnet

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The manuscript by Dr. Maurice Balke et al. entitled, "A short-term in vivo model for giant cell tumor of bone" describes the testing of a new model for the studying of GCT. Here, the authors demonstrate that a well vascolarized GCT tumor developed on a chick chorio-allantoic membrane (CAM) that resemble the three histologically different components of the original tumor.

Major Compulsory Revisions

- Results. “Histological and Immunohistochemical findings” paragraph. “The giant cells reacted for CD68 and CD51, indicating that these cells were osteoclasts; there were also CD68+ macrophages in the mononuclear component...”. CD68 and CD51 can both be expressed by macrophages that derive from the same precursor of osteoclasts. Therefore, it is hard to establish if the staining is really associated to an osteoclast-like or to a macrophage-like phenotype. Moreover, the figure showing results on CD68 and CD51 is not included in the paper, although it is commented in the result section. According to the aim of the manuscript, the demonstration of the formation of osteoclasts rather than reactive macrophages in the GCT model is quite striking. In fact, the authors state that osteoclast cultures form GCT are quite difficult to obtained, and therefore an alternative model is needed. On the contrary, both monocytes and stromal-like cells can be easily isolated and characterized, as documented by several papers. The figure showing the CD68 and CD51 staining should be added, and additional demonstrations that osteoclasts rather then macrophages were obtained are needed.

- Results. “Histological and Immunohistochemical findings” paragraph. “Tumor giant cells frequently contained more than five nuclei in the original tumors but were smaller and contained fewer nuclei in the cultured samples...” also macrophages can have 4-5 nuclei. See above for comments.

- Discussion, Third paragraph. “Histologically the GCTs in our model closely resembled the pulmonary metastases of GCT.....the latter were generally less numerous and less multinucleated than in the original tumor, a common morphological finding in GCT lung nodules”. The association between GCT model in CAM and GCT lung metastases is rather speculative. Moreover, the cited reference reports that in lung metastases there are few multinucleated cells in respect to the primitive tumor, but the number of nuclei are not mentioned.

- Discussion, Third paragraph. “Thus the model would appear to simulate the early phase of tumor seeding, one of the initial steps in the development of a
metastasis or local recurrence”. This statement is speculative. Moreover, results obtained about the similarities with lung nodules are contradictory in respect to the aim of the study. In fact, the authors state that their GCT model is more similar to the lung nodules rather than to the primitive tumor. GCT metastases are very rare in GCT patients, and are not the best model to study GCT biological and molecular mechanisms that develops in the bone microenvironment.

Minor essential revision
- References. Add the year to the reference n. 49.
- Figures. In figure 4a and B, the authors show indicate with arrows the multinucleated cells because they are poorly evident.

Discretionary Revisions
- It is hard to be convinced that multinucleated cells can resist to a cycle of freezing and defrosting. It could be interesting to investigate if the multinucleated cells in the GCT specimens are newly formed and derived from implanted human monocytes or if they are rather derived directly from the cell suspension obtained from the tumor biopsies. Suggested experiment: the TRAP staining associated to nuclear staining of defrosted cells cultured in vitro at 3-4 days after seeding.
- To demonstrate that among the obtained cells in the GCT in the CAM there are osteoclasts, a cell suspension isolated from the tumor mass, as previously done for tumor biopsies, could be seeded for a bone resorption assay.

Level of interest: An article of limited interest

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