



Department of Molecular and Comparative Pathobiology

Re: Rebuttal letter.

December 12, 2020

Dear Dr. Nguyen,

We appreciate the review of our manuscript” **A syngeneic orthotopic osteosarcoma Sprague Dawley rat model with amputation to control metastasis rate**”. The manuscript was revised based on the following reviews.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
2. Please provide an email address for each author.
3. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text:
 - a) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.
 - b) Discuss maintenance of sterile conditions during survival surgery.
 - c) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
 - d) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.
4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all

commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Eppendorf; Hope Micro-Max X-ray processor; Faxitron Mx20 etc

5. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
6. 1: Please cite a reference (passaging, sub-culturing, trypsinization, counting etc) for the cell culture part.
7. 2.1.1: what signs do you look for when monitoring depth of anesthesia?
8. 2.1.5: please specify the vital signs and temperature (both body and of the heating pad) so readers can replicate your protocol.
9. Please include steps (even if you cite references) stating that western blotting can be performed.
10. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.
11. Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend.
12. Please do not abbreviate journal names in the reference list.
13. Please include the molecular marker in the western blot (Figure 8).

We addressed all suggestions and made corrections following the paper's policy and the suggestions.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This model describes a model for osteosarcoma in the rat, which can be used for studying osteosarcoma metastasis. This is clinically highly relevant, given the devastating consequence of osteosarcoma metastases in human subjects. The fact that it is a syngeneic model is especially important for testing immunotherapy based drugs in a model system. The protocol described is very

detailed and well documented and many useful practical tips are provided.

Major Concerns:

Major concerns

1. A major hallmark of osteosarcoma is the production of osteoid. However the histological pictures in Fig 5 and 6 do not show any clear osteoid. Could a more representative picture of tumor histology be shown?

We added new histopathology photographs as suggested.

2. Could the authors provide documentation on the size of the metastases in amputated vs. non-amputated animals? Is there a marked difference?

No pattern in the size or number of metastasis was observed and this note was added to the manuscript.

3. It is intriguing that late passage UMR106 cells are more aggressive upon implantation. The authors may speculate why this is observed.

The reason for this is not known but we can only speculate that cells in culture could develop mutations that favor metastasis. This was added to the manuscript.

Minor Concerns:

The authors say that there is tumor invasion in the cortical bone in Fig 5C. But this is actually more likely to be woven bone occurring after healing of the wound made for orthotopic injection.

We agree with you and provided new histopathology photographs to show lytic areas in cortex with a woven bone reaction.

Reviewer #2:

Manuscript Summary:

The article describes a protocol for inducing the metastatic osteosarcoma model in Sprague-Dawley rats. The procedure and the results presented in the paper are convincing. The steps of the procedure are clearly explained, and most of the critical steps are highlighted. However, I have some concerns regarding some points of the procedure and did not find several important details. Please find my concerns below.

Major Concerns:

Isoflurane concentration in these experiments is unusually low. From my experience, it is hard to induce

anesthesia with 2-3% isoflurane. Also, 1.5% for maintenance during invasive surgery is quite a low concentration. No data on the gas is provided. There is also no data on scavenging systems used. It would be highly recommended to clearly state that using scavenging system is essential. The authors do not address the issue of sterile technique in surgeries. They should explain whether they observed infections and how to identify them. I did not also find any mention of the administration of antibiotics.

The procedure should define humane endpoints.

The necropsy procedure should include confirmation of animal's death.

The list of materials and equipment is very short and not very detailed; for example, isoflurane vaporizer, scavenging system, equipment for monitoring of the animals, most of the basic materials for surgeries, surgical tools, sutures etc.

The description of results is mixed with interpretations and description of future directions. I would suggest rewriting this section and including some information from this section in the Discussion.

We appreciate the suggestions and incorporated all into the revised manuscript.

Minor Concerns:

Line 59 - The authors state that they hypothesize that this model is pre-clinically relevant. However, they do not test this hypothesis. After this statement, they refer to the model's characteristics or well-known data to support this hypothesis. I would suggest modifying this sentence and stating clearly the aim of the study.

Line 61 - I would suggest to clearly state that UMR106 cells originate from SD rats.

Line 68 - "this rat model is poised" - I would suggest rewriting this sentence

Line 69 - I would suggest rewriting this sentence - this is not really a conclusion

Minor spell check is required.

Line 89 - "leading to some advantages" - please be more specific

Line 237 - please provide a route of administration

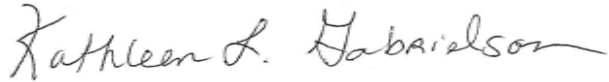
Line 252 - please refer to radiography rather than to the equipment's name (the same concerns other references to this technique). The details of the machine should instead be put in the materials table.

Line 255 - no anesthesia procedure described

We have followed each suggestion made by the reviewers and editor and our manuscript is much improved. We revised the abstract, introduction, methods, results, legends and discussion. We have also added more panels to figures with new photographs of both bone and lung cancer in response to the reviews. We also added molecular weight markers to the western blots.

We sincerely appreciate the opportunity to resubmit our manuscript.

Sincerely,

A handwritten signature in black ink that reads "Kathleen L. Gabrielson". The signature is written in a cursive, flowing style.

Kathleen Gabrielson, DVM, Ph.D.

Associate Professor

Molecular and Comparative Pathobiology, Pathology and Oncology