UNIVERSITY of IOWA
CARVER COLLEGE OF MEDICINE
University of Iowa Health Care

Department of Surgery

Po Hien Ear, PhD
Research Scientist
University of Iowa Roy J. and Lucille A.
Carver College of Medicine
375 MERF, Room 4235
Iowa City, IA 52242
Tel. (319)335-8983
E-mail: pohien-ear@uiowa.edu

July 24th, 2019

RE: Rebuttal for Editorial Comments of Manuscript JoVE60303 "Establishment and characterization of small bowel neuroendocrine tumor organoids,"

Dear Dr. Bajaj,

Thank you for expressing interest regarding our manuscript. We have submitted a revised copy of the manuscript addressing all the editorial and reviewers' comments along with figures in Illustrator format. We apologize for the delay! We wanted to make sure that we addressed all the reviewers' comment thoroughly. The current manuscript is now 10 pages long. We hope the editorial team and reviewers will like our modifications and recommend this manuscript for publication.

Please see below for our point by point responses to the Editorial comments.

Sincerely,

Po Hien Ear, PhD
Research Scientist
James Howe Laboratory
Department of Surgery
University of Iowa
375 Newton Rd,
MERF Room 4235
Iowa City, IA 52242
Lab phone: (319) 335-8983

Rebuttal for Editorial Comments:

Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Thanks for pointing this out. We have performed the revision.

2. 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: Matrigel, Abcam-45179, Abcam-32127, VECTASHIELD, Vector Laboratories, Jackson ImmunoResearch, etc

Ok, all removed.

3. Please ensure that the short abstract/summary is no more than 50 words.

Corrected, it's 49 words now.

4. Please expand the Introduction to include all of the following:
a) A clear statement of the overall goal of this method

Added
b) The rationale behind the development and/or use of this technique

added

c) The advantages over alternative techniques with applicable references to previous studies

added
d) A description of the context of the technique in the wider body of literature.

added

e) Information to help readers to determine whether the method is appropriate for their application. We updated the introduction with more information.

5. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee. We've included a statement with IRB protocol number. We included the statement and IRB protocol number.

6. Please include a single line space between each step, substep, and note in the protocol section. We included the single lines for spacing.

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Yes, we double checked and inserted a few Notes in the protocol.

8. The Protocol should contain only action items that direct the reader to do something. Yes, confirmed.

9. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps of the protocol should only contain 2-3 actions per step. We double checked.

10. Please ensure you answer the "how" question, i.e., how is the step performed? Yes, we checked.

11. 1.1: How and from where the tumors were obtained? We included a statement saying that tumors were resected from patients in the operating room, brought to the surgical pathology core facility for analysis, and a fraction of the tumors were cut into 5 mm piece for storage in DMEM/F12 medium.

Do you perform any wash steps before storing? No, resected tumors were simply cut into smaller pieces and stored in DMEM/F12.

Do you immediately transfer it to DMEM, or this can be stored at -80 C? No storage at -80^{o}C. Resected tumor samples are stored at 4^{o}C in DMEM/F12 for up to 3 hrs prior to processing. Overnight storage will decrease the yield of viable organoids.

How do you transfer the tumor samples to the lab-based settings? In 50 mL conical tubes.

What is the general size of the tumors obtained? Do you make small pieces before transferring to DMEM? Cut tumors into 5 mm cubes and use 3 or more pieces for processing to ensure enough tumor cells can be obtained.

12. 3, 4: After how many days do you perform the characterization and rapamycin treatment? We have SBNET spheroids growing in culture for up to 9 months now and they still express the NET markers (see new Figure 7). SBNET spheroids can be used for drug treatment experiment at any timepoint. We tested our SBNET spheroids that were in culture for 2 months with rapamycin treatment for 5 days.

Citations for the antibody used as the marker for organoid culture? These antibodies have been used in other cancers and tissue sections but not for neuroendocrine tumors. Hence, our pathologist team has included the detailed conditions IHC in the new Section 5.

13. 3.1: Is it one organoid per tube? We plate ~ 1000 to 10000 spheroids per well (96-well plated).

14. 3.11, 4.6: What is the mangnification used? 10X, 20X, 40X for immunofluorescence and 400X for immunohistochemistry. Images were cropped with appropriate scale bars in ImageJ.

15. 4. Do you perform DMSO control as well? Yes, new figure with 4 conditions.

16. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. We have highlighted the 2.5 pages of protocol for the filmable content.

17. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]." We haven't published these elsewhere. No copyright permission is needed.

18. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations: We've edited the Discussion section.
a) Critical steps within the protocol

Added

b) Any modifications and troubleshooting of the technique

We added ROCK inhibitor to the culture media to slightly improve cell survival of SBNET spheroids after thawing. In addition, we switched from calling our SBNET "organoids" to "spheroids" since Reviewer#3 pointed out that our 3D cultures are not heterogenous.

c) Any limitations of the technique

Added

d) The significance with respect to existing methods

Added

e) Any future applications of the technique

Added *in vivo* model as future application.

19. Please sort the materials table in alphabetical order.

We sorted the materials/equipment.

20. The signed ALA is for the UK only. Please print and sign the attached Author License Agreement (ALA). Please then scan and upload the signed ALA with the manuscript files to your Editorial Manager account.
We are from the USA. No ALA needed.