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Dr. Phillip Steindel,
Review Editor
JoVE

Dear Phillip,

Thank you for your positive response to our manuscript entitled "*Measuring Real-time Drug Responses in Organotypic Tumor Tissue Slices*". We would especially like to thank you and the editorial board members for the comments, and all the reviewers for their thoughtful consideration and insightful recommendations.

We are now submitting a revised version of the manuscript that addresses the points raised by the reviewers. I hope that we have responded in a satisfactory manner and that the revised manuscript is now suitable for publication in JoVE.

Thank you for your consideration. I look forward to your response

Sincerely,

Taran Gujral, PhD

Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have carefully proofread the manuscript to correct for any grammatical errors.

2. Please ensure that the manuscript is formatted according to JoVE guidelines–letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.

We have changed the formatting of our manuscript to follow the description above.

3. Please provide at least 6 key words or phrases.

We have included more key words in the Keywords section.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of 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: RealTime-Glo, IVIS

We have rephrased KimWipes, RealTIme-Glo and IVIS to appropriate words.

Protocol:

1. If necessary, 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.

An ethics statement has been provided before the numbered protocol.

2. There is a 10 page limit for the Protocol, but there is a 2.75 page limit for filmable content. If revisions protocol to be more than 2.75 pages, please highlight 2.75 pages or less of the Protocol (including headers 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.

Our protocol section is approximately 3 pages long. We have highlighted filmable content of our procedure in yellow.

3. For each protocol step/substep, 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. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

We have carefully proofread our manuscript to follow the instructions and made appropriate changes highlighted with 'track changes'.

Figures:

1. 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 have included the phrase "These data have been published previously[10]." in the legend of Fig. 3 (B).

2. Please provide 1 file per figure (3 in total). Please remove 'Fig. 1' etc. from the Figures themselves.

Updated figure files are attached.

Acknowledgment and Disclosures:
1. Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

Disclosure section was included before the Reference section.

References:
1. Please do not abbreviate journal titles.

We have revised the name of the journal titles to full terms.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.
2. Please remove trademark (™) and registered (®) symbols from the Table of Materials.

We have removed ™ and ® from the revised Table of Materials.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This contribution from Gujral et al provides an important new method and another highly needed comparative data set for the discovery of small molecule potential as cytotoxic agents versus cancer models. The screening of cancer cell lines versus approved and investigational drugs is a key first step in the development of a new drug or the repurposing of existing therapies. However, for solid tumors the use of cultured cells may not alway recreate the key disease features that predict in vivo outcomes. The tumor slice method, while sacrificing throughput, is likely going to allow for a greater predictive outcome.
Major Concerns:
None
Minor Concerns:
It would be good to see additional models/outcomes. But others will surely follow.

We appreciate your positive comments on our protocol. We hope our protocol will be utilized by many researchers studying other types of cancers and models.

Reviewer #2:

Manuscript Summary:

The authors state that the assay is applicable for medium to high throughput assays. However, I don't see high throughput as a possibility (thousands to millions of compounds). Therefore, I would say low to medium throughput.

We appreciate the reviewers careful reading and thoughtful comments to improve our manuscript. We agree that the tissue slice culture system itself is incapable of screening on the order of millions of compounds. However, we expect screening of hundreds of inhibitors is possible if the system becomes automated, or by utilizing our computational modeling-based inhibitor screening (PNAS, 2014, Nat Comm, 2017 and our manuscript in preparation). Therefore, we hope to keep this phrase as is.

Major Concerns:

It would be much more compelling if a more extensive characterization of the culture conditions would be added. For example, do slices maintain proliferation (e.g. by doing EdU staining), how much apoptosis develops (e.g. TUNEL staining), is tissue morphology still intact (HE staining), does immune cell content change over time (specific immunostainings), etc. I understand that this manuscript mainly describes the procedures, but without these baseline parameters it is very difficult for readers to decide whether it would be a good assay to be used.

We and our collaborators have extensively characterized organotypic tissue slices using histological characterizing from pancreatic ductal carcinoma (Jiang et al., 2017) and liver tissue (Wu at al., 2018), proliferation by staining Ki67+ (Jiang et al., 2017), as well as apoptosis by caspase-3 (Jiang et al., 2017), and immune cell population (Jiang et al., 2017, Wu at al., 2018, Sivakumar et al., 2019). Proliferation of cells in tissue slices by EdU or equivalent proliferation markers was also evaluated by several other publications during optimization of tissue slice culture conditions (Naipal et al., 2016, Nagaraj et al., 2018, Vesci et al, 2015). We have cited most of these references in our manuscript. Therefore, we consider tumor tissue slice culture to have been well-characterized, and it is not necessary to repeat in this protocol. In the context of this manuscript, we would like to introduce a new method for real-time evaluation of drug efficacy of tissue slices.

References

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